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Mechanisms of the dilator action of cryptotanshinone on rat coronary artery

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Abstract

In this study, we have investigated the actions of cryptotanshinone, an active, lipophilic component of the medicinal herb danshen (*Salvia miltiorrhiza*), on rat isolated coronary artery rings precontracted with 1 μM 5-hydroxytryptamine (5-HT) and its action compared to the ethanol-extractable fraction of the herb. Extraction of the ethanol-soluble fraction from danshen provided a yield of 1%. The amount of cryptotanshinone determined in this ethanol extract was 3.682%, and it was 6 times more potent than the extract in relaxing 5-HT-precontracted coronary artery rings; IC_{50} values were 2.65±0.15 μg/ml and 15.82±1.07 μg/ml, respectively. Involvement of endothelium-dependant mechanisms in their dilator effects were investigated by pretreatment of the artery rings with a cyclooxygenase inhibitor flurbiprofen (10 μM), a nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM), a muscarinic receptor antagonist atropine (100 nM), and by mechanical removal of the endothelium; none of these procedures produced a significant change on their dilator actions. Involvement of endothelium-independent mechanisms was investigated in endothelium-denuded artery rings pretreated with a β-adrenoceptor antagonist propranolol (100 nM), an adenylyl cyclase inhibitor 9-(tetrahydro-2-furanyl)-9H-purine-6-amine (SQ22536, 100 μM), a guanylyl cyclase inhibitor 1*H*-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one (ODQ, 10 μM), and a potassium channel inhibitor tetraethylammonium (TEA, 100 mM); these also produced no change on their dilator actions. The possible involvement of Ca^{2+} channels was investigated in artery rings incubated with Ca^{2+}-free buffer and primed with 1 μM 5-HT for 5 min prior to adding $CaCl_2$ to elicit contraction. The danshen ethanol extract (100 μg/ml) abolished the $CaCl_2$-induced vasoconstriction, whereas, cryptotanshinone (30 μg/ml) produced 59% inhibition. These findings suggest their vasorelaxant effects are independent of pathways mediated by the endothelium, muscarinic receptors, β-adrenoceptors, adenylyl cyclase, and guanylyl cyclase, whereas, inhibition of Ca^{2+} influx in the vascular smooth muscle cells is important for their vasodilator actions. The high vasodilator potency and the quantity of salvianolic acid B contained in danshen ethanolic extract suggest it is an important constituent in this medicinal herb.

Keywords: Cryptotanshinone; Danshen; Calcium channel; Coronary artery; (*Salvia miltiorrhiza*)

1. Introduction

The potential value of herbal medicine has been rediscovered in recent years. This is exemplified by the encouragement of the World Health Organization's Traditional Medicines Strategy to incorporate herbal medicines into medicinal care. An example of such standard herbal medication is danshen, the dried root of *Salvia miltiorrhiza* belonging to the family of Labiatae. This herbal medicine has been approved for clinical use in China for treatment of cardiovascular diseases that include angina pectoris, myocardial infarction, and stroke (Ji et al., 2000). The cardio-protective efficacy of danshen has been confirmed in animal ischaemia–reperfusion experiments (Fung et al., 1993; Ji et al., 2000; Kuang et al., 1996; Wu et al., 1993; Zhou and Ruigrok, 1990). The mechanism may involve the ability of danshen to enhance antioxidant defense enzymes activities to decrease or abolish the production of free radicals (Ji et al., 2003). Danshen has also been shown to attenuate the increase in intracellular Ca^{2+} induced by anoxia-reoxygenation in isolated ventricular myocytes, which would decrease the transformation of xanthine oxidase from xanthine dehydrogenase to reduce the production of oxygen free radicals (Cao et al., 2003). In addition, danshen lowered the viscosity of whole blood, accelerated electrophoresis of red blood cells, and improved peripheral circulation (Chen 1981).

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The content of danshen can be separated into lipophilic and hydrophilic fractions. Its lipophilic fraction contains more than 30 diterpenoid tanshinones; the major active constituents include tanshinone I, IIA, B, cryptotanshinone, dihydrotanshinone I, methylenetanshinone, and isotanshinone IIA (Cheng et al., 1990). Earlier studies have shown that cryptotanshinone could be metabolized to tanshinone IIA in vivo (Xie and Shen, 1983: Xue at al., 1999). These two diterpenoids and tanshinone I have attracted the attention of many clinicians because of their diverse biological properties, such as anti-inflammatory, antibacterial, anti-tumour, anti-oxidative, anti-mutagenic, and anti-platelet aggregation activities (Mosaddik, 2003; Ryu et al., 1997; Cao et al., 1996, Wang et al., 1989). Such diverse properties have encouraged the use of danshen and its diterpenoid ingredients in clinical management of a plethora of diseases including hepatitis, menstrual disorder, miscarriage, diabetes, and chronic asthmatic bronchitis (Zhang et al., 2006).

The putative active components of the hydrophilic fraction of danshen are salvianolic acid B, danshensu, lithospermic acids, protocatechuic acid, and rosemarinic acid (Chan et al., 2004; Kamata et al., 1993; Kohda et al., 1989; Liu et al., 1992). Many of the salvianolic acid derivatives and diterpenoids in danshen are reported to have beneficial effects in stroke and ischaemic diseases (Adams et al., 2006). Their vasodilator and hypotensive properties probably contributed to these effects (Kamata et al., 1993; Lei and Chiou, 1986; Li et al., 1990). Recently, we have elucidated the mechanisms of the dilator action of the hydrophilic fraction of danshen and two of its ingredients, salvianolic acid B and danshensu, in rat coronary blood vessels. All of them were found to produce vasorelaxation by inhibiting Ca^{2+} channels with a minor component mediated by the opening of K^+ channels (Lam et al., 2006b, 2007). In the present study, we have extracted the lipophilic fraction from a danshen herb by ethanol extraction and quantified cryptotanshinone content in this extract. We have also compared the actions of this extract with cryptotanshinone on coronary arteries isolated from rats.

2. Materials and methods

Experiments were performed on male Sprague-Dawley rats (250–300 g) bred and kept by the Laboratory Animal Services Centre of the Chinese University of Hong Kong. All experiments were performed under licence from the Government of the Hong Kong SAR and endorsed by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong.

2.1. Extraction of ethanolic fraction of danshen

Dried danshen herb purchased from Eu Yan Sang Ltd. (Hong Kong) was used in the present study. For extraction of the ethanolic faction, 200 g of the danshen herb was cut into small pieces and boiled in 250 ml 95% ethanol under reflux condition. After 1 h, the mixture was filtered and the filtrate was collected. A further 250 ml ethanol was added to the danshen residue and boiled for one more hour. The filtrate was collected and combined with the previous filtrate sample, allowed to cool at room temperature, and dried by a rotary evaporator below 5 °C. A brown residue was obtained and re-dissolved in ethyl acetate. The ethyl acetate layer was collected and dried by a rotary evaporator.

2.2. Identification and quantification of cryptotanshinone

HPLC was performed with the danshen ethanol extract to quantify and confirm the presence of cryptotanshinone in the sample. Authentic standard of cryptotanshinone was purchased from Chengdu Congon Bio-tech Co. Ltd. (China). The danshen ethanol extract was dissolved in methanol at a concentration of 500 μg/ml. HPLC analysis was performed in triplicate; each with 50 μl of the sample. Cryptotanshinone was separated on an Agilent Zorbex Eclipse XDB-C18 5 μm (4.6×150 mm) with a XDB-C18 guard column. A gradient elution of A (1.5% acetic acid in water, pH 4.7) and B (acetonitrile) was used at a flow rate of 1 ml/min, commencing with 55% A and 45% B, rising to 60% B on 3 min, then to 80% B on 19 min. Detection was by an Agilent 1100 Series HPLC with diode-array detector at 260 nm. Standard curve for cryptotanshinone was linear between 5 to 100 μM (0.74 to 14.8 μg). The identity of cryptotanshinone was confirmed by comparison of its retention time to the authentic cryptotanshinone standard, and quantification of cryptotanshinone was based on the peak area ratio of cryptotanshinone over the internal standard (α-naphthoflavone) against the respective concentration of cryptotanshinone.

2.3. Isolation and mounting of blood vessels

Male Sprague-Dawley rats (250–300 g) were killed by cervical dislocation and exsanguinated. From each animal, the left anterior descending coronary artery was carefully removed and placed in Krebs–Henseleit solution: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.18, $NaHCO_3$ 25, glucose 10 mM. Adherent adipose and connective tissue were removed. Vessel rings of 1.5 mm length were cut from each artery and they were then mounted with 20 μm steel wires to separate 5 ml tissue baths of an integrated myograph system (Danish Myo Technology Model 619 M) for tension recording. The tissue baths were filled with Krebs–Henseleit solution at 37 °C, and aerated with 95% O_2/5% CO_2 to maintain pH at 7.4; washout was by draining and replacement of bathing solution from a syringe. Tension signals were relayed to a MacLab 4 amplifier and saved to a Macintosh PowerMac computer system (sampling rate 100/s).

2.4. Experimental protocols

During the initial one hour equilibration period, the vessel rings were stretched until the resting tension held steady at 0.5 g. The preparations were then checked for reproducible contractile response to 1 μM 5-HT. Integrity of the endothelium in each vessel ring was confirmed by an adequate relaxant response (>70%) to 1 μM acetylcholine on the 5-HT-precontracted tone. The preparation was then washed with Krebs–Henseleit solution and 1 μM 5-HT was again added to establish stable contractile tone. Subsequently, concentration–response curves of danshen ethanol extract and cyrptotanshinone were obtained by cumulative addition of these agonists on to the vessel ring at 10 min intervals.