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Anti-myocardial ischemia effect of *Syringa pinnatifolia* Hemsl. by inhibiting expression of cyclooxygenase-1 and -2 in myocardial tissues of mice

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ABSTRACT

Ethnopharmacological relevance: The peeled stem of *Syringa pinnatifolia* Hemsl. (SP) is a traditional medicine in Inner Mongolia, China. The powder form of SP has been widely used for hundreds of years to relieve "He-Yi" related myocardial ischemia independently or in a traditional Chinese medicine preparation.

Materials and methods: SP was extracted with 95% and 80% ethanol. Chemical profiling was performed using HPLC-DAD and IT-TOF-ESI-MS analyses. Myocardial ischemia was produced by ligation of the left anterior descending (LAD) coronary artery to evaluate the anti-myocardial ischemia effect of SP. Male C57BL/6 mice were randomly divided into six groups (n = 10 per group): a sham group, a model group, groups pretreated with SP at three dosages (20 mg/kg, 40 mg/kg, and 80 mg/kg, intragastrically), and a positive control group (acetylsalicylic acid, ASA, 53 mg/kg, intragastrically). Echocardiography was performed to determine heart function by measuring ejection fraction and fractional shortening. The levels of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) in serum, and 6-keto-$PGF_1\alpha$ and TXB_2 both in plasma and in protein homogenate of myocardial tissue were also measured. The levels of cyclooxygenase (COX)-1 and -2 in the heart tissue and their expressions in mouse myocardial tissue were determined using Western blot and an immunofluorescence assay, respectively. Inflammatory cell infiltration and collagen deposition changes in the myocardial ischemic tissue were observed by pathological examination.

Results: Intragastric pretreatment with SP produced a dose-dependent increase in cardiac function. SP at 80 mg/kg significantly improved the EF ($p < 0.001$) and FS ($p < 0.01$) compared with the model group, as well as the levels of serum CK-MB and LDH decreased obviously ($p < 0.001$), approaching those in the sham group. Besides, an obvious reduction in inflammatory cells infiltration and collagen deposition in the infarcted myocardial tissue was shown in each SP treatment group. In addition, SP increased 6-keto-$PGF_1\alpha$ and decreased TXB_2 levels in the plasma, whereas the opposite pattern was observed in the protein homogenate from the myocardial tissues at the infarction edge, but keeping balance the ratio of 6-keto-$PGF_1\alpha$ and TXB_2, which is better than ASA in plasma. The mechanisms is associated with the downregulated expressions of COX-1 ($p < 0.05$) and COX-2 ($p < 0.001$).

Conclusions: Ethanol extract of SP has a protective effect against myocardial ischemia *via* down regulation of COX-1 and COX-2 expression and by adjusting the ischemia-induced imbalance between 6-keto-$PGF_1\alpha$ and TXB_2. This study shows substantial evidence to support the clinical application of SP and indicates that such medicine has great potential for treating ischemia-induced heart disease.

Abbreviations: SP, *Syringa pinnatifolia* Hemsl.; IHD, ischemic heart disease; LAD, left anterior descending; ASA, acetylsalicylic acid; LVEDs, left ventricular end-systolic diameter; LVEDd, left ventricular end-diastolic diameter; EF, ejection fraction; FS, fractional shortening; COX, cyclooxygenase; CK-MB, creatine kinase-MB; LDH, lactate dehydrogenase; TXA_2, thromboxane A_2; PG, prostaglandin; AA, arachidonic acid; HE, hematoxylin-eosin; NSAID, nonsteroidal anti-inflammatory drugs

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1. Introduction

Ischemic heart disease (IHD) has become one of the leading causes of death worldwide over the past decades (Hanes et al., 2015; Wang and Sun, 2014). One crucial pathological process, myocardial ischemia, occurs when there is insufficient blood flow and oxygen supply to the heart muscle, resulting in myocardial infarction, coronary heart disease, angina pectoris, and heart failure (Kang et al., 2007; Shirai, 2004). Consequently, ischemia-induced acute myocardial infarction has emerged as the most important cause of death in the industrialized world, causing 600,000 deaths every year in America alone (Sodha et al., 2009).

The underlying mechanisms involved in pathogenesis of myocardial ischemic injury are complex, including myocardial fibrosis and progressive left ventricular remodeling (Yin et al., 2008), generation of reactive oxygen species that directly induce cell death and apoptosis by injuring the cell membrane (Zhou et al., 2012). There is increasing evidence to suggest that inflammation plays a significant role in the underlying mechanisms of myocardial ischemic injury (Frangogiannis et al., 2002; Saito et al., 2004). Inflammation is at least partially mediated by prostaglandins (PGs), and PGs are mediated by the rate-limiting enzyme cyclooxygenases (COXs)-1 and -2. These two COX isoforms mediate the enzymatic conversion of arachidonic acid (AA) to PGH_2 and other PG metabolites (Sharma and Jawad, 2005). The COX-1 isoenzyme is expressed constitutively and initiates the production of PGs that regulate gastrointestinal function and thromboxane A_2 (TXA_2) activity, which stimulates platelet aggregation and maintains normal hemostasis. COX-2 is regulated by inflammatory factors such as cytokines or tumor promoters, leading to the production of PGI_2 and other prostaglandins that cause inflammation, pain, and fever (Lefkowith, 1999; Sirois and Richards, 1992). AA is released from membrane phospholipids in large amounts after ischemic events, the COXs then catalyze the conversion of AA into the intermediate PGH_2, which is then metabolized to produce PGI_2, TXA_2, PGE_2, $PGF_2\alpha$, and PGD_2 (Yu et al., 2014).

Currently, the clinical drugs for IHD include nonsteroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid (ASA) (Amer et al., 2010; Dubois et al., 1998; Guirguis-Blake et al., 2015) and selective COX-2 inhibitors such as celecoxib (Zhao et al., 2012). However, ASA can inhibit COX, which catalyzes the synthesis of cyclic endoperoxides from AA to produce PGs, resulting in gastric ulcers, untoward platelet functions, and other side effects (Dubois et al., 1998; Simon, 1996). Selective COX-2 inhibitors are associated with an increased risk of hypertension and thromboembolic complications, including myocardial infarction (Grosser et al., 2006; Sowers et al., 2005). Therefore, it is increasingly important to discover and develop comprehensive cardioprotection regimes, including investigating the efficacy of drugs derived from natural resources, particularly those with a long clinical history of use in traditional medicines.

Syringa pinnatifolia Hemsl. (SP), belonging to the family Oleaceae, is a deciduous shrub mainly distributed amongst the shrubs and spinneys of Helan Mountain, Inner Mongolia, China. The peeled stem, named “Shan-chen-xiang” in Chinese, has been employed as a traditional medicine for hundreds of years in Inner Mongolia, China, for the treatment of cardiovascular symptoms, asthma, pain, and fever (National Pharmacopoeia Committee, 1998; Nei, 1987; Qi, 2002). Traditionally, SP has been used in powder and it was believed that the therapeutic effects arise from the purple resins in the stem wood (State Administration of TCM, 2004). Consequently, the essential oil was generally considered to be the major pharmacologically effective ingredients for cardioprotection against myocardium ischemia, hypoxia, and platelet aggregation (Yan et al., 2010). However, we proposed that the essential oil may not contribute to all therapeutic effects of SP and the bioactive ingredients may contain more constituents, such as lignans and sesquiterpenes, that occur in the stem wood (Su et al., 2015a, 2015b). Therefore, in this study, we investigated the anti-myocardial ischemia effects of the extract from the peeled SP stems. Heart function changes were evaluated using echocardiography in a mouse model of myocardial ischemia. To confirm the cardioprotective effect of SP, the levels of CK-MB and LDH in serum and the levels of 6-keto-$PGF_1\alpha$ and TXB_2 in plasma and protein homogenates from the edge of the myocardial ischemic region were measured. Furthermore, COX-1 and COX-2, which are associated with inflammation, were measured by western blot and immunofluorescent staining assay. In brief, this study demonstrates the cardioprotective effects of ethanol extract of SP against myocardial ischemia and provided evidence that the preliminary mechanism of SP action is *via* regulation of COX-1 and COX-2.

2. Materials and methods

2.1. Instrument, reagents and HPLC-DAD-IT-TOF-MS analysis

The liquid chromatographic analysis was performed on a Shimadzu LC system (Shimadzu, Kyoto, Japan) consisting of two LC-20 CE_{XR} solvent delivery units, a SIL-20AC_{XR} autosampler, a CTO-20AC column oven, a SPD-M20A diode array detection (DAD) module, a DGU-20A_{3R} degasser, and a CBM-20A controller. A hybrid ion trap/time-of-flight mass spectrometer (Shimadzu) equipped with an electrospray ionization (ESI) source was connected to LC system *via* a PEEK tube (0.13 mm i.d.) to perform high-resolution tandem mass spectrometry. The chromatographic separation was performed on a Zorbax SB C_{18} column (250 × 4.6 mm, 5 μm, Agilent). Acetonitrile (A)–0.1% aqueous with formic acid (B) were used as mobile phase and the flow rate was set at 1.0 mL/min with a gradient program as follows: 0–8 min, 10–19% A; 8–32 min, 19–24% A; 32–40 min, 24–26% A; 40–70 min, 26–43% A; 70–90 min, 43–55% A; 90–110 min, 55–90% A; 110–125 min, 90% A. Roughly 20% portion of the effluent was introduced into the ESI source by splitting the effluent *via* two PEEK tubes with length ratio of 1:4. UV absorption over 190–400 nm was recorded by DAD unit and the detection wavelength was set at 280 nm. The injection volume was set at 10 μL. At the end of each run, the initial composition of mobile phase (10% A) was permitted to re-equilibrate the whole system for 10 min

The optimized operating conditions of high resolution mass spectrometer (HRMS) analysis were as follows: positive mode; nebulizer gas (N_2) flow, 1.5 L/min; CDL temperature, 200 °C; heat block temperature, 200 °C; detector voltage, 1.40 kV; interface voltage (+), 4.5 kV; ion accumulated time, 10 ms; repeat times, 2; collision energy was set at 50% for MS^2, MS^3 and MS^4; pressure of ion trap, 1.9e−002 Pa; pressure of TOF region, 1.2e−004 Pa; scan range *m/z* 100–1000; precursor ion isolation, 3.0000 Da. An automatic scan mode was used in the HPLC-ESI-MS^n analysis.

LC-MS grade acetonitrile is product of Fisher Scientific (Fair-Lawn, NJ, USA). Ultrapure water was prepared in our own laboratory by Milli-Q plus System (Millipore, Bedford, MA, USA). Analytical-grade solvents used for sample preparation are products of Beijing Chemical Factory (China).

2.2. Preparations of SP extraction

The stems of *S. pinnatifolia* Hemsl. were collected in July 2013 from Alxa League' Inner Mongolia, China, and authenticated by Prof. Suyile Chen (Alashan Mongolian Hospital, Inner Mongolia). A voucher specimen (SP201307S) was deposited in the Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine. The stems of SP (35 kg) were extracted with