Journal of Ethnopharmacology 155 (2014) 240–247

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Effect of the total saponins of *Aralia elata* (Miq) Seem on cardiac contractile function and intracellular calcium cycling regulation

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ARTICLE INFO

Article history:
Received 15 December 2013
Received in revised form
14 May 2014
Accepted 18 May 2014
Available online 27 May 2014

Keywords:
Total saponins
Intracellular calcium concentration
Positive inotropic effect (PIE)
Cardiomyocyte

ABSTRACT

Ethnopharmacological relevance: Total saponins of *Aralia elata* (Miq) Seem (AS) from the Chinese traditional herb Longya *Aralia chinensis* L. can improve cardiac function, although the active mechanism remains poorly understood. The present study aimed to determine the direct effect of AS on cardiac function in dogs and the effects on Ca^{2+} transient and contractions in isolated rat cardiomyocytes.
Material and Methods: In anesthetized dogs, hemodynamic indexes and myocardial oxygen consumption were determined before and after AS was administered. In isolated adult rat cardiomyocytes, contractile and intracellular Ca^{2+} properties were determined simultaneously in real time by using an IonOptix MyoCam system.
Results: Our results showed that AS directly induced a positive inotropic effect and improved coronary blood flow and energy metabolism, indicating that AS induced a beneficial effect to treat myocardial ischemia/reperfusion injury. Moreover, AS increased sarcomere shortening, maximal velocity of shortening/relengthening ($\pm dL/dt$), amplitude of $[Ca^{2+}]_i$ transients and SERCA activity in a concentration-dependent manner. PKCε was also activated after the cells were treated with AS.
Conclusion: These findings revealed the positive inotropic effect of AS on canine myocardium and isolated rat cardiomyocytes. This effect was possibly associated with an increase in amplitude of the $[Ca^{2+}]_i$ transient and PKCε-dependent signaling pathway.

1. Introduction

Aralia elata (Miq) Seem, which belongs to Araliaceae family, is a shrub widely distributed in Northeastern China, Far East Russia, Japan, and Korea (Li and Lu, 2009). The bark and roots have been traditionally used as a tonic, anti-arrhythmic, anti-arthritic, antihypertensive and anti-diabetic agent in traditional Chinese medicine (Xu et al., 1997; Li and Lu, 2009). *Aralia elata* is also a well-known adaptogenic plant used in Russia and the extract of Aralia was officially approved as a tonic for therapeutic use in 1957 (Wojcicki et al., 1977; Yance and Tabachnik, 2007). Aralia species contain some ginseng-like triterpenoid saponins (Aralosides) and the effect produced by aralosides is similar to that of panaxosides from ginseng (Baranov, 1982), which probably contributes to Aralia's ability to increase energy, strengthen body, and improve the body's hypoxia ability in regard to the cardiovascular system and to other parameters (Sololov et al., 1971; Baranov, 1982; Yance and Tabachnik, 2007). Total saponins of *Aralia elata* (AS), the main pharmacologically active ingredient extracted from *Aralia elata* (Xu et al., 1997), have been shown to stimulate heart activity (Sokolov, 1965; Xu et al., 1997), possess anti-myocardial ischemic and anti-hypoxic activities (Deng et al., 1988; Wen et al., 2005; Sun et al., 2006), exhibit a strong anti-arrhythmic effect (Maslov and Guzarova, 2007; Arbuzov et al., 2009; Maslov et al., 2009) and have protective effect against diabetic cardiomyopathy (Xi et al., 2009). However, the direct effects of AS on cardiac function in canines and its underlying mechanism of cardiac contractility remain largely unknown.

Cardiac contraction is regulated by the excitation-contraction (EC) coupling (Fearnley et al., 2011). Ca^{2+} cycling has an important function during this process. Contraction is initiated by Ca^{2+} entry into cardiac myocytes via L-type Ca channels (LTCC). This process

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http://dx.doi.org/10.1016/j.jep.2014.05.024
0378-8741/

subsequently triggers a greater release of Ca^{2+} from the sarcoplasmic reticulum (SR) via ryanodine receptors, thereby providing Ca^{2+} to stimulate the myofilaments to induce contraction. In cardiac relaxation, Ca^{2+} that enters the cell via LTCC is transported out of the cell mainly by the sarcolemmal Na^{+}/Ca^{2+} exchanger (NCX). Ca^{2+} released from the SR is reuptaken by SR Ca^{2+} ATPase (SERCA), thereby providing available Ca^{2+} for the next contraction cycle. This whole event determines the contraction and relaxation of the myocytes (Cheng and Lederer, 2008). However, the relationship between the cardiac effect of AS and calcium cycling has not been clarified. Studies have yet to determine whether or not the mechanism of the positive inotropic activity of AS is caused by an increase in the amplitude of $[Ca^{2+}]_i$ transient during EC coupling. Moreover, studies have shown that protein kinase C (PKC), a large family of serine/threonine protein kinases, is crucial in regulating contractility and Ca^{2+} cycling in the heart (Rogers et al., 1990; Pi and Walker, 2000). Whether or not PKC are involved in AS-regulated cardiac contraction remain unknown.

The present study aimed to examine the effect of AS on cardiac function in canines and evaluate the effect of AS on EC coupling by simultaneously determining the effects of AS on cell sarcomere shortening and intracellular Ca^{2+} transients in isolated adult rat myocytes. We also studied the involvement of SERCA and PKC in these effects.

2. Materials and methods

2.1. Plant material

The roots of AS were collected from Jilin Province of China in September 2010. The samples were identified by Professor Zhong-Kai Yan (Academy of Chinese Medical Sciences of Jilin Province). A voucher specimen (No. 20100920) was deposited in the same department.

2.2. Extraction and isolation of AS

The roots of AS were refluxed thrice with 70% alcohol solution for 1.5 h at each time. The solution was filtered, subjected to a macroporous resin column, and eluted successively with deionized water, 20% and 80% ethanol. The solutions eluted by water and 20% ethanol were discarded. The remaining 80% ethanol solution was collected and evaporated to dryness under reduced pressure to obtain the total saponins (Zhang et al., 2013).

2.3. UPLC analysis

The total saponin (0.01 g) was dissolved in methanol (5 mL × 2) under ultrasonic irradiation. The sample was passed through a 0.22 μm filter prior to injection. Known amounts of the reference standards were weighed and dissolved in methanol to prepare the solutions at approximately 1 mg/mL. The standards were diluted 50-fold to prepare the working solutions before injection. The samples were separated on an Acquity UPLCTM system (Waters Corp., USA). Chromatographic analysis was conducted using a Waters phenyl column (100 mm × 2.1 mm i.d., 1.7 μm) at 25 °C and a flow rate of 0.4 mL/min. The mobile phase consisted of acetonitrile (A) and water with 0.05% formic acid (B); the eluting gradient was used as follows: 0–3 min, 2.0–11.0%; 3–10 min, 11.0%; 10–16 min, 11.0–20.0%; 16–18 min, 20.0–24.0%; 18–22 min, 24.0%; 22–22.5 min, 24.0–30%; 22.5–26 min, 30.0%; 26–33 min, 30.0–80.0%; and 33–35 min, 80.0–100.0%.

2.4. Mass spectrometry

Mass spectrometry detection was performed on a Synapt G2 MS system (Waters Corp., USA) equipped with an ESI source. Two data acquisition modes, or MSE, were selected to investigate precursor ions and product ions. Nitrogen gas was used for nebulization. The detection mode of the flying tube was selected as "V" pattern. The positive ion spectra of the column eluates were recorded at a range of m/z 100–1500. The optimized conditions of the ESI source were listed as follows: capillary voltage, 2.5 kV; sampling cone voltage, 40 V; extraction cone voltage, 3.0 V; ESI source temperature, 120 °C; desolvation temperature, 450 °C; cone gas flow, 30 L/h; desolvation gas flow, 800 L/h; collision gas flow, 0.5 mL/min; collision energy for MSE acquisition mode, 4.0 eV for low energy scan and 15–40 eV for high energy scan; and dynamic adjustment of the fragmentor voltage ranged from 25 V to 40 V for the MS/MS acquisition mode. The lock mass compound was leucine enkephalin (m/z 556.2771), and the interval scan time was 0.02 s. Masslynx 4.1 (Waters Corp.) software was used to control this instrument.

2.5. Animals

Male and female adult mongrel dogs (15–17 kg) were obtained from Norman Bethune University of Medical Science. Male Sprague-Dawley rats (180–200 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China. The animals were housed under standard laboratory conditions (25 ± 1 °C, 60% humidity, and 12 h photoperiod) and provided free access to sterile food and water. All of the procedures were approved by the Laboratory Animal Ethics Committee of the Institute of Medicinal Plant Development, Peking Union Medical College with the registration number: #IMPLAD2012112207.

2.6. Measurement of blood pressure, heart rate, ventricular function, and coronary artery blood flow in anesthetized dogs

Twenty-four animals were randomly divided into four groups: a control group, two AS groups (30 and 60 mg/kg) and a Di-ao-xin-xue-kang capsule (Di-ao capsule) group (86 mg/kg) as a positive drug, with $n=6$ in each group. The dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) and then a ventilator was set up. A catheter connected to a pressure transducer was introduced into the femoral artery to record blood pressure and heart rate. After an incision was produced on the neck, a catheter connected to a pressure transducer was introduced into the left ventricle (LV) via the right carotid artery. LV systolic pressure (LVSP), end-diastolic pressure (LVEDP), and the maximal rates of pressure increase ($+dp/dt$) and decrease ($-dp/dt$) were measured or calculated for each group. A left lateral thoracotomy was performed in the fourth intercostal space, the pericardium was incised, and the heart was suspended in a pericardial cradle. A probe was connected to the left circumflex coronary artery, and coronary blood flow (CBF) was recorded using an electromagnetic blood flow meter system (Nihon Konden, Japan). Myocardial flow (MF) was expressed as mL/min per 100 g of myocardium supplied by a coronary artery. All of these parameters were recorded at different times (15, 30, 45, 60, 90, 120, 180, 210, and 240 min) before and after different concentrations (30 and 60 mg/kg) of AS were duodenally administered.

2.7. Measurement of myocardial oxygen consumption in dogs

Blood was collected from the femoral artery and the coronary sinus at different times (15, 45, 60, 90, 120, and 240 min) before and after AS was administered for blood gas analysis (DH-1300 blood gas analyzer, Nanjing Analytical Instrument