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Extracts from *Astragalus membranaceus* limit myocardial cell death and improve cardiac function in a rat model of myocardial ischemia

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ABSTRACT

Ethnopharmacological relevance: The root of *Astragalus membranaceus,* known as "huang-qi", is one of the most widely used Chinese herbal medicines for the prevention and treatment of myocardial ischemic diseases. However, the mechanisms governing its therapeutic effects are largely unknown.
Aims of the study: The aims of the present study were to investigate the cardioprotective effect of the root extract of *Astragalu membranaceus* (EAM) in myocardial ischemia and to explore its underlying mechanisms in ROS-mediated signaling cascade *in vivo* and *in vitro.*
Materials and methods: The saponins in EAM were analyzed using HPLC. The tests for the cardioprotective effects of EAM and its mechanisms were performed *in vivo* and *in vitro. In vivo*, the rat model of persistent myocardial ischemia was produced by occlusion of the left anterior descending (LAD) coronary artery. *In vitro*, the cardiomyocyte model of oxidative stress was mimicked by the direct free radical donor, H_2O_2.
Results: In vivo, the increased myocardial infarct size and the increased serum levels of lactate dehydrogenase (LDH), creatine kinase isoform MB (CK-MB), and cardiac troponin (cTnI) were significantly decreased by pre-treatment with EAM. Moreover, cardiac function, as assessed by $\pm dP/dt$, left ventricular developed pressure (LVDP), and left ventricular end-diastolic pressure (LVEDP), was dramatically improved. An oxidative stress biomarker, malondialdehyde (MDA), was reduced, and the antioxidant enzyme superoxide dismutase (SOD) was induced. *In vitro*, H_2O_2-triggered myocardial cell death and cytoplasm Ca^{2+} overload were blocked by treatment with EAM. Furthermore, the K_{ATP} channel blocker (5-HD, glibenclamide) blocked the anti-apoptotic protective effect of EAM on cardiomyocytes injured by H_2O_2.
Conclusions: The cardioprotection of EAM was manifested as a protection of tissue structure and as a decrease in serum markers of ischemic injury. The mechanisms underlying the EAM-mediated protective effects may involve improving cardiac function, attenuating the oxidative injury *via* a decrease in MDA, a maintenance in SOD, and a reduction in free radical-induced myocardial cell injury. Additionally, EAM enhanced the myocardial cell viability *via* arresting the influx of Ca^{2+} to block cell death and opening mitochondrial K_{ATP} channels to reduce cell apoptosis.

1. Introduction

Myocardial ischemia is one of the cardinal pathological features of ischemic cardiovascular disease, which is the leading cause of death worldwide. The pathogenesis of injury in myocardial ischemia has many mechanisms. The anatomic changes and the distinctions of biochemical markers for ischemic myocardial cell injury and death have been well characterized. The cardiac dysfunction is generated in myocardial ischemia, and the loss of energy substrates in myocardial ischemia leads to the generation of reactive oxygen species (ROS) (Levraut et al., 2003); the high levels of ROS are detrimental (Kloner et al., 1989) and can induce a variety of cardiomyocyte abnormalities (Fu et al., 2007).

Based on the ROS that plays a role in the pathogenesis of myocardial ischemia, an increasing number of studies have been conducted to study the related mechanisms for the prevention and treatment of myocardial ischemia. Mitochondria are a likely source for excessive ROS generation. The ROS hasten lipid peroxidation, DNA damage, and other direct cellular injuries that result in apoptosis in cells (Halliwell and Aruoma, 1991; Yoshikawa et al., 2006). Accordingly, successful antioxidant interventions targeted to reduce the levels of ROS offer insights into delaying or preventing the onset of ischemic heart disease (Zhu et al., 2004). Recent studies have suggested that activation of mitochondrial ATP-sensitive potassium (mito-K_{ATP})

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channels and recovery of Ca^{2+} homeostasis have a cardioprotective effect against myocardial ischemic injury (Ferranti et al., 2003; O'Rourke, 2004; Li et al., 2012).

The root of *Astragalus membranaceus* (*Radix astragali*), known as "huang-qi", is a traditional Chinese medicine that has been widely used to treat conditions associated with tissue ischemia, including cardiovascular and cerebrovascular diseases. The beneficial effects of *Astragalu membranaceus* and its extracts on myocardial ischemic diseases have been extensively investigated. Laboratory studies have shown that *Astragalu membranaceus* and its extracts are capable of protecting myocardial cells from damage that is induced by isoproterenol (Xu et al., 2008), viruses (Yang, 1991), autoimmune responses (Zhao et al., 2008), and they have a potential protective effect against daunorubicin cardiotoxicity, which has been shown to trigger free radical release and apoptosis in cultured neonatal cardiomyocytes (Luo et al., 2009). Astragaloside IV, a saponin composition of *Astragalu membranaceus*, has a strong protective effect against ischemia reperfusion injury (Zhang et al., 2006). However, little information is available regarding the preventative effects of the extract of *Astragalu membranaceus* (EAM), which include two types of major and active constituents in *Astragalu membranaceus, i.e.*, saponins and flavones, on acute myocardial ischemia. The underlying and detailed mechanisms of the cardioprotective effects of *Astragalu. membranaceus* remain largely unknown.

The aims of our present study were as follows: (i) to assess whether pre-treatment with EAM produces cardioprotective effects in the model of acute myocardial ischemia, using anatomical indices and biochemical markers; (ii) to evaluate whether the cardiac function of EAM treatment is improved using the hemodynamic parameters; (iii) to explore the effects of EAM on oxidative stress and antioxidant defense in the myocardium during ischemia *in vivo* or *in vitro*; and (iv) to determine whether the inhibition of calcium influx and the activation of mito-K_{ATP} are, in part, involved in the antioxidant defense.

2. Methods

2.1. Preparation of EAM and high-pressure liquid chromatography (HPLC) analysis

After Professor Peng-Fei Tu authenticated the origin of the roots of *Astragalu membranaceus*, the extract was prepared using the following method. The dried roots of *Astragalu membranaceus* were powdered and extracted three times using gently boiling distilled water (70 °C, 2.5 h per extraction). The extract was combined, filtered and concentrated; the polysaccharides in the combined extracts were precipitated by adding 80% ethanol (EtOH) to the paste. The supernatant was collected, evaporated under reduced pressure, and filtered. The filtrates were adsorbed on a porous polymer gel HPD-600 column and successively eluted with 70% EtOH. The fraction of 70% EtOH elution was collected, concentrated under vacuum and sprayed to dryness to obtain the extract of the roots of *Astragalu membranaceus* (EAM). The yield ratio of EAM was 1%. EAM was stored in a refrigerator (4 °C) until use; the time limit for EAM storage was 1 year.

Analysis of astragalosides of EAM was performed using a liquid chromatograph (Series 1100, Agilent Technologies, Palo Alto, CA, USA), consisting of a dual pump, an autosampler, an ELSD (Alltech Associates, Deerfield, IL, USA), a ZORBAX ODS C_{18} column and a guard column using HP ChemStation software (Agilent Technologies). The column temperature was maintained at a constant 25 °C, and the mobile phase flow rate was 0.8 ml/min. The mobile phase consisted of acetonitrile (solvent A) and H_2O (solvent B), which were applied in a gradient elution as follows: 0–5 min, 18–20% A; 5–35 min, 20–31% A; 31–45 min, 31–37% A; 45–60 min, 37–38% A; and 60–75 min, 38–46% A. The drift tube temperature for the ELSD was set at 110 °C, and the nebulizing gas flow rate was 3.0 L/min. To analyze flavanoids of EAM, an ultraviolet (UV) detector was used, and the mobile phase flow rate was 0.8 ml/min. The mobile phase consisted of acetonitrile (solvent A) and H_2O (solvent B), which were applied in a gradient elution as follows: 0–40 min, 3–30% A; 40–85 min, 30–85% A; 65–70 min, 85% A; 70–75 min, and 85–3% A. UV spectra were monitored at 275 nm.

2.2. Animals

Male Sprague-Dawley (SD) rats, 16 weeks old, were obtained from the Laboratory Animal Breeding and Research Center of Peking University Health Science Centre, China. The certificate number of these rats was SCXK2002-2001. The present study was approved by the Institutional Animal Care and Use Committee and conducted according to the guidelines for the Care and Use of Laboratory Animals at Peking University, which followed the guidelines of the National Institutes of Health (NIH). The rats were kept in plastic cages in an environmentally controlled room (22 ± 2 °C, 45–60% humidity) with free access to pellet food and water on a 12-h light/dark cycle.

2.3. Animal model and drug administration

The rat model of persistent myocardial ischemia was produced by ligation of the left anterior descending coronary artery, as described previously with minor modifications (Stanton et al., 2000; Samsamshariat et al., 2005). We anesthetized rats with sodium pentobarbital (50 mg/kg). After tracheal intubation, the rats were ventilated using a respirator (HX300, Chengdu, China) with room air (tidal volume, 3 ml/100 g; respiratory rate, 60 cycles/min). A thoracotomy was performed in the fourth intercostal space under sterile conditions. The heart was exposed, and the left coronary artery was ligated 2–3 mm from its origin with a 6–0 Prolene suture. The sham group underwent thoracotomy and cardiac exposure without coronary ligation. The surviving rats were maintained on standard rat chow.

The rats were randomly selected and placed into the following six experimental groups ($n = 10$ in each group): Group 1, the sham-operated control group (sham), where the rats underwent all of the surgical procedures, but the suture passing under the coronary artery was not tied; Group 2, the ischemia group, where the operated rats were administered 0.9% NaCl orally (10 ml/kg); and Groups 3–6, the treatment group, where the rats received EAM at different doses (100 mg/kg, 200 mg/kg, 400 mg/kg, or 600 mg/kg) orally twice a day for 7 times before ischemia. The operation to induce ischemia was performed thirty minutes after the last dose.

2.4. Determination of infarct size and hemodynamic measurements

Infarct size was determined by staining the viable myocardia with triphenyltetrazolium chloride (TTC). The heart was rapidly excised, and the entire left ventricle was sectioned into five 2-mm slices in a parallel plane from the base to the atrioventricular groove. The slices were subsequently incubated in 1% TTC for 10 min at 37 °C to distinguish the viable and necrotic tissue. The viable tissue was stained red, whereas the infarct tissue remained pale. Each slice was traced along the border between the infarct and the viable area. The infarction area ratio was defined as the area of the infarct divided by the total left ventricle area and expressed as a percentage (Maczewski and Mackiewicz, 2007).

Hemodynamic measurements were obtained before sacrificing the animals according to the method described by Feng et al. (2001). After the rats were anesthetized with sodium pentobarbital