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Original article

The cardioprotective effect of total flavonoids on myocardial ischemia/reperfusion in rats

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ABSTRACT

The flowers of *Abelmoschus manihot (L.) Medic* is a traditional Chinese medicine used for the treatment of ischemic diseases. The present study is to investigate whether total flavones (TA) of extracted from *Abelmoschus manihot L. Medic* has the potential cardioprotective effect on myocardial ischemia/reperfusion (I/R) damage in rats. The index of myocardial injury, inflammatory biomarkers and NLRP3-related parameters were measured, respectively. The results demonstrated that compared to I/R group, TA reduced myocardial infarction area, declined serum creatinine kinase (CK), lactate dehydrogenase (LDH) levels, attenuated serum interleukin-6 (IL-6), IL-1β and tumour necrosis factor (TNF-α) production. Moreover, TA markedly enhanced the activities of superoxide dismutase (SOD) and reduced the amounts of malondialdehyde (MDA) in I/R rats. In addition, TA reduced myocardial I/R induced injury in rats by inhibiting NLRPR3 inflammasome. Thus, it is assumed that TA could significantly improve myocardial I/R injury in rats partially through suppressing NLRP3 activtion.

1. Introduction

Heart disease is one of the major health problems worldwide. Extensive researches focused on studying cardioprotective therapies throughout the last decade [1,2,3], among which myocardial ischemia/reperfusion (I/R) attracts lots of attention attributes to its complex pathophysiological process. Early reperfusion is critical for tissue salvage, while prolonged reperfusion may leads to additional myocardial injury termed as “reperfusion injury” which is serve than that produced by ischemia alone [4]. IR injury results in serious acute or chronic myocardial damage, including myocardial ultrastructural alterations, remodeling, systolic and diastolic dysfunction [5]. Therefore, it is essential to comprehensively understand the mechanisms of I/R injury and seek for novel therapeutic strategies to treat myocardial ischemia/reperfusion damage.

The inflammatory reaction is one of the most important links in the pathogenesis of myocardial ischemia reperfusion injury [6]. The inflammatory stresses caused by cell infiltration reflects the severity of I/R injury. Proinflammatory cytokines such as TNF-α, IL-6 and IL-1β contribute a lot to the development of myocardial I/R [7,8]. As the core of the inflammatory response, NLRP3 inflammasome might provide new targets for the therapy of various inflammatory diseases. Therefore, it is supposed that NLRP3 may be involved in the pathogenesis and pathological processes of myocardial ischemia/reperfusion.

Abelmoschus manihot (L.) Medic is an edible hibiscus of the Malvaceae (Mallow) family and its flower, used as a staple of folk medicine in Eastern Europe and Asia, possesses various pharmacological effects including fertility control, childbirth mitigation, lactation stimulation and menorrhagia resistance [9]. Phytochemical studies indicated that the major constituents of total flavonoids (TA) extracted from flowers of *Abelmoschus manihot (L.) Medic* were quercetin, hyperin, isoquercetin, quercetin-3′-O-glucoside, hibifolin and myricetin, etc. [10,11]. Pharmacological researches have suggested that TA possess a varity of biological activities such as antiinflammatory, antibacterial, antioxidant, anticonvulsant, cardioprotective and neuroprotective effects [12].

Currently, no available study evaluated the effects of TA on I/R injury and conduct further reserches. Therefore, the present study was designed for the first time to investigate the potential cardioprotective effects of TA on I/R injury in rats and explore its possible mechanisms.

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2. Materials and methods

2.1. Reagents

TA was obtained from Nanjing university of Chinese medicine. The enzyme-linked immunosorbent assay (ELISA) kits for determination of IL-6, IL-1β and TNF-α were produced by Nanjing KeyGEN Biotech. CO., LTD. (Nanjing, China). CK, LDH, MDA and SOD kits were provided by Jiancheng Bioengineering Institute (Nanjing, China).All antibodies were purchased from Cell Signaling Technology Inc (Beverly, MA, USA).

2.2. Preparation of TA

The fresh flowers of *Abelmoschus manihot (L.) Medic* were purchased from Simcare Drug Store (Nanjing, China). The plant material was identified and authenticated as flowers of *Abelmoschus manihot (L.) Medic* by Professor Jianwei Chen, a pharmacognosist, from Nanjing University of Chinese Medicine (Nanjing, China).

The flowers were dried in the shade and pulverized in a mechanical grinder. Dried flowers powder (2.5 kg) were extracted three times with ethanol (80%, v/v) under reflux for 2 h. After filtration and combination of the filtrates, they were concentrated to dryness in the rotary evaporator, a crude ethanol extract (750 g) is collected. The crude ethanol extract was redissolved in hot water and filtrated. The water solution was subjected to polyamide gel column chromatography eluted with gradient mixtures of ethanol and distilled water. The ethanol elution were collected and evaporated under reduced pressure. Ethanol elution yielded total flavonoids (70 g) following solvent removal under vacuum. The yield was 2.8%. Phytochemical analysis of the plant extracts has previously been carried out using standard procedures and reported [13].

2.3. HPLC analysis of TA

HPLC was performed on a Waters Acquity HPLC system (Waters, Milford, MA, USA), consisting of a binary solvent delivery system, an on-line degasser, an autosampler and a photo-diode array detector (PDA) system. An ACQUITY UPLCTM BEH C18 (2.1 × 100 mm I.D., 1.7 mm, Waters, Milford, USA) column was used for all the analyses. The mobile phase composed of A (0.1% formic acid, v/v) and B (acetonitrile) with a gradient elution: 0–3 min, 90–70%A; 3–6 min, 70–0%A; 6–8 min, 0%A; 8–10 min, 90%A. The flow rate of the mobile phase was 0.4 mL min-1and the column, auto-sampler temperature were maintained at 30 and 10 °C, respectively.

2.4. Animals

All the studies were performed using male Spraguee Dawley rats weighing 180 ± 220 g. The animals were fed with a normal rodent chow diet and had free access to tap water *ad libitum*. Besides, rats were housed at a constant temperature and relative humidity under a regular 12 h light/12 h dark schedule.

2.5. Experimental protocol and drug administration

Rats were randomly assigned to five groups: sham group, I/R group, I/R + TA (40 mg/kg) group and I/R+ TA (80 mg/kg) group. The surgical procedures were performed as described previously by Bhindi et al. [14]. The successful establish of the myocardial ischemia/reperfusion model was verified by regional cyanosis of the myocardium and ST-segment elevation of electrocardiogram (ECG) [15]. To examine cardiac function and myocardial infarct size, reperfusion was prolonged to 24 h. At 4 h of reperfusion, TA was administered in one more doses. The experiments were conduct on non-diseased hearts without abnormal ECG. Except accidental deaths due to anesthesia or failed surgery, the number of rat in each group was as follows: sham group (control, n = 10), I/R group (n = 8), I/R + TA (40 mg/kg) group (n = 8), I/R + TA (80 mg/kg) group (n = 9).

2.6. Electrocardiographic (ECG)

The ECGs was recorded using the BL-420S Biologic Function Experiment system (Chengdu, China).

2.7. Assay of myocardial infarct area

After reperfusion, myocardial infarct size was determined by means of a double-staining technique and a digital imaging system (infarct area/area at riskx100%) [16]. Meanwhile, coronary blood flow was again blocked and Evans blue (2%, 4 mL) was injected by the rapid distribution of the right ventricle into the body. The heart was quickly removed and set aside at −20 °C for Western blot and pathology analysis. The heart was cut into 1 mm slices, placed in 1% 2, 3, 5-triphenyltetrazolium chloride (TTC) solution, incubated for 15 min, and then placed in 4% formaldehyde solution overnight. Evans blue stained area (blue staining, non-ischemic area), TTC stained area (red staining, ischemic area) and non TTC stained areas (white, infarct area) were analyzed with a digital imaging system by computer. Myocardial infarct area (infarct area/area at risk%, INF/AAR%) was calculated.

2.8. Levels of serum LDH and CK-MB

Myocardial cellular damage was evaluated by measuring serum LDH and CK-MB levels. 3 h after reperfusion, serum CK-MB and LDH activities were measured spectrophotometrically according to the manufacturer's instructions.

2.9. Levels of serum SOD and MDA

SOD and MDA levels were measured by a rate assay using an RT-9600 Semi-automatic Biochemical Analyzer (ShenZhenLeiDu life Science, LLC). All the experimental procedures were performed according to the manufacturers' instructions.

2.10. Cytokine detection

The levels of IL-6, IL-1β and TNF-α in serum were measured using ELISA kits according to the manufacturer's instructions. The concentrations of the cytokines were quantified by referring to standard curves.

2.11. Histological examination of myocardium

Immediately after the sacrifice of rats, hearts were removed and fixed in 10% formalin solution. The heart tissue was processed for sectioning and staining by standard histological methods. Sections (5 mm, Leica RM 2125, Germany) from the left ventricle were stained with hematoxylin and eosin (H&E) and examined by light microscopy (Nikon, Tokyo, Japan) at 200× magnification.

2.12. Western blot

Myocardial tissue was minced and homogenized in ice-cold RIPA buffer containing 0.1% phenylmethylsulfonyl fluoride. The dissolved proteins were collected from the supernatant after centrifugation at 12000*g* for 20 min. Protein concentrations were