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Protective roles of cornuside in acute myocardial ischemia and reperfusion injury in rats

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ABSTRACT

Cornuside is a secoiridoid glucoside isolated from the fruit of *Cornus officinalis SIEB. et ZUCC*. In this study, we investigated the anti-myocardial ischemia and reperfusion (I/R) injury effects of cornuside *in vivo* and elucidated the potential mechanism. Rat models of myocardial I/R were induced by coronary occlusion followed by reperfusion or by Isoproterenol (ISO), treatment of rats with cornuside (20 and 40 mg/kg, *i.v.*) protected the animals from myocardial I/R injury as indicated by a decrease in infarct volume, improvement in hemodynamics and reduction of myocardial damage severity. Treatment with cornuside also attenuated polymorphonuclear leukocytes (PMNs) infiltration, decreased myeloperoxidase (MPO) activity in the heart, lowered serum levels of pro-inflammatory factors and reduced phosphorylated IκB-α and nuclear factor kappa B (NF-κB) levels in the heart. Additionally, cornuside was shown to have remarkable antioxidant activity and inhibited ISO-induced myocardial cell necrosis. Thus, cornuside appeared to protect the rat from myocardial I/R injury by acting as an anti-inflammatory agent. These findings suggested that cornuside may be used therapeutically in the setting of myocardial I/R where inflammation and oxidant injury are prominent.

Introduction

Myocardial ischemia and reperfusion (I/R) is associated with a strong inflammatory response (Entman et al., 1991). Endothelial injury is thought to play a critical role in the pathogenesis of myocardial I/R injury by setting the stage for adherence of neutrophils to the vascular endothelium and subsequent development of an inflammatory response. Many studies have indicated that nuclear factor kappa B (NF-κB) plays a key role in myocardial I/R injury (Frantz et al., 2007). Activation of NF-κB has been reported in post-ischemic reperfusion tissue (Kis et al., 2003). Suppression of polymorph nuclear leukocytes (PMNs) infiltration and inhibiting NF-κB activation diminishes myocardial I/R damage and potentially offers myocardial protection (Kim et al., 2009).

Cornuside (Fig. 1) is a secoiridoid glucoside isolated from the fruit of *Cornus officinalis SIEB. et ZUCC*, a traditional oriental medicine for treating inflammatory diseases and invigorating blood circulation. Crude extract of the fruit of *C. officinalis* has been found to be have pharmacological actions such as anti-neoplasm, anti-inflammatory, hepatoprotection, anti-diabetic nephropathy and anti-sepsis effects (Xu and Hao, 2004; Chang et al., 2004; Jiang et al., 2009). Studies have also shown that cornuside suppresses the expression of cytokine-induced pro-inflammatory and adhesion molecules in human endothelial cells (Kang et al., 2007). Based on both the traditional therapeutical effect of *Cornus officinalis SIEB. et ZUCC* on blood circulation and the demonstrated anti-inflammatory effects of cornuside, we hypothesized that cornuside may protect the heart from the injury induced by I/R and the subsequent inflammatory response. The aim of this study was to investigate the effects of cornuside on rat models of myocardial I/R and explore its potential mechanisms of cardioprotection.

Materials and methods

Materials

Cornuside (Purity > 99.0%, CAS no.: 131189-57-6, molecular formula: $C_{24}H_{30}O_{14}$ molecular weight: 542.49) and total *Cornus officinalis* extract were provided by Department of Chemistry, College of Marine Life Science, Ocean University of China. Trolox

Abbreviations: I/R, ischemia–reperfusion; PMNs, polymorph nuclear leukocytes; Tn-T, troponin T; LAD, left anterior descending coronary artery; LVSP, left ventricular systolic pressure; MPO, myeloperoxidase; NF-κB, nuclear factor kappa B; LDH, lactate dehydrogenase; CK-MB, creatine kinase-MB; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6; MDA, malondialdehyde; SOD, superoxide dismutase; GPx, glutathione peroxidase.

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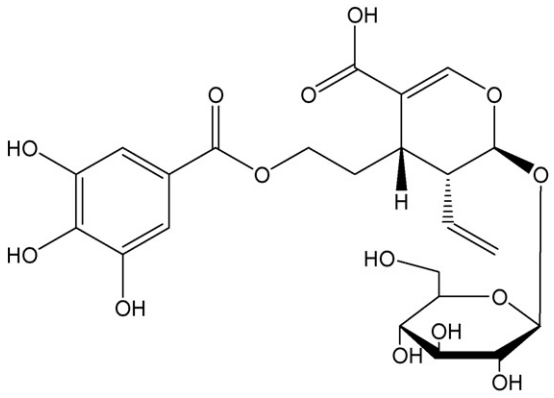

Fig. 1. Chemical structure of cornuside.

(Merck, Shanghai. Molecular formula: $C_{14}H_{18}O_4$, molecule weight: 250.297); troponin T (Tn-T), enzyme-linked immunosorbent assay (ELISA) kits and myeloperoxidase (MPO) kits were purchased from Maisha Biology Company (Shanghai, PR China). Tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) ELISA kits were purchased from Xitang Biology Technology Company (Shanghai, PR China). Polyclonal rabbit anti-mouse phosphor-NF-κB and phosphor-IκB-α antibodies were purchased from Biosynthesis Biotechnology Company (Beijing, PR China). Lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) kits were purchased from Zhongsheng Biotechnology Company (Beijing, PR China).

Animals

Adult male Sprague–Dawley rats (250–280 g, body weight) were housed individually under constant temperature (22 ± 2 °C) and humidity with a 12 h light/dark cycle and had free access to chow and water. All experimental procedures were approved by the institutional Animal Ethics Committee.

Determination of the content of cornuside in total Cornus officinalis extract

Analyses were performed using an Agilent 1100 HPLC system (Agilent Technologies, USA) with diode array detector. Detection wavelengths were set at 218 nm for cornuside determination. An Agilent C_{18} column (250 mm × 4.6 mm, 5 μm) was used with a flow rate of 1.0 ml/min. The injection volume was 10 μl and the column temperature was maintained at 30 °C. Mobile phase was composed of 0.1% aqueous phosphoric acid and acetonitrile (86:14).

Isoproterenol (ISO) induced myocardial necrosis in rats

Sixty rats were divided into six subgroups (n = 10 per group): (I) non-myocardial ischemia rats; (II) ISO rats received saline alone; (III) ISO rats received intravenous injection (*i.v.*) of cornuside 10 mg/kg; (IV) ISO rats received *i.v.* of cornuside 20 mg/kg; (V) ISO rats received *i.v.* of cornuside 40 mg/kg; (VI) ISO rats received *i.v.* of total *Cornus officinalis* extract 80 mg/kg. All groups except the sham group animals were administered ISO (150 mg/kg *s.c.*) for 2 consecutive days. Animals were treated with cornuside daily for 3 days, starting 30 min after *s.c.* injection of ISO. At 72 h after *s.c.* ISO treatment, blood was drawn from retro orbital veins and serum was prepared by centrifugation after clotting for lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) measurements.

Induction of myocardial I/R injury

Rats were anesthetized with sodium pentobarbital (40 mg/kg) through intraperitoneal injection. Myocardial I/R operation was induced according to a previously published procedures (Jiang et al., 2010). Briefly, coronary artery ligation was achieved with a gab fixed onto the left anterior descending coronary artery (LAD). A 7-0 silk suture was passed underneath the LAD (2–3 mm inferior to the left auricle) and tied. Significant ECG changes, including widening of the QRS complex and elevation of ST segment were the indicators of successful coronary occlusion. The chest was closed in layers, and the respirator weaned when the animal recovered with spontaneous breathing.

One hundred and ninety rats were divided into three groups, the first group and the second group include six subgroups (n = 10 per subgroup): (I) non-myocardial I/R rat, the silk suture crossed without ligation and the rat did not incur I/R; (II) I/R rats received saline alone; (III) I/R rats received intravenous injection (*i.v.*) of cornuside 10 mg/kg; (IV) I/R rats received *i.v.* of cornuside 20 mg/kg; (V) I/R rats received *i.v.* of cornuside 40 mg/kg; (VI) I/R rats received *i.v.* of total *Cornus officinalis* extract 80 mg/kg. The third group was composed of the same experimental subgroups as described above, but also included an additional subgroup of Trolox treatment 10 mg/kg. This extra subgroup was used as a positive control to compare the antioxidant potency of cornuside. After reperfusion for 5 min, all experimental animals received drug treatment (*i.v.*) at the indicated dosage. Cornuside, total *Cornus officinalis* extract and Trolox were dissolved in sterilized saline to make stock solutions and appropriate dilutions were made according to the dosages required. The first group of animals was used for hemodynamics evaluation, infarct size and serum levels of Tn-T, TNF-α and IL-6 determination. The second group was used for histopathological analysis. The third group was used to determine the level of malondialdehyde (MDA) and superoxide dismutase (SOD), glutathione peroxidase (GPx) and MPO activities as well as for Western blots analysis.

Determination of hemodynamics

At 24 h after I/R, all rats were anesthetized with sodium pentobarbital (40 mg/kg) through intraperitoneal injection and a Millar vessel was inserted into the left ventricular cavity via the right common carotid artery. The pressure was transduced and amplified by a pressure transducer. Left ventricular systolic pressure (LVSP) and $\pm dp/dt_{max}$ were recorded and programmed using a biotic signal collection and processing system (BIOPIC, American).

Determination of serum levels of Tn-T, TNF-α and IL-6

At 24 h after I/R, blood samples were collected. Serum levels of Tn-T, TNF-α and IL-6 were measured using ELISA kits according to the manufacture's instructions. IL-6 level was expressed as pg/ml while TNF-α and Tn-T levels were expressed as ng/ml.

Analysis of myocardial infarction

Myocardial infarction was determined according to a previously described method (Takano et al., 1998), with specific areas determined using the Adobe Photoshop computer program. The nonischemic area, area at risk, and infarct area of each tissue slice were separated, weighted and calculated as a percentage of corresponding area multiplied by the weight of the slice.

Determination of the MDA level, SOD, GPx and MPO activities in myocardial tissue of the left ventricle

At 24 h after I/R, the MDA level, SOD, GPx and MPO activities in ischemic myocardial tissues were measured using kits according to