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Cardioprotective effects of salidroside on myocardial ischemia–reperfusion injury in coronary artery occlusion-induced rats and Langendorff-perfused rat hearts

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ABSTRACT

Background/objectives: The current study was designed to investigate the protective role of salisroside on rats through the study of energy metabolism homeostasis and inflammation both *in ex vivo* and *in vivo*.
Methods: Energy metabolism homeostasis and inflammation injury were respectively assessed in global ischemia of isolated hearts and coronary artery ligated rats.
Results: Excessive release of cardiac enzymes and pro-inflammatory cytokines was inhibited by salidroside in coronary artery occlusion-induced rats. ST segment was also restored with the treatment of salidroside. Triphenyltetrazolium chloride staining (TTC) staining and pathological analysis showed that salidroside could significantly alleviate myocardial injury *in vivo*. Accumulated data *in ex vivo* indicated that salidroside improved heart function recovery, which was reflected by enhanced myocardial contractility and coronary flow in isolated hearts. The contents of ATP and glycogen both *in ex vivo* and *in vivo* were restored by salidroside compared with those in the model group. Besides, the expressions of p-AMPK, PPAR-α and PGC-1α in rats and isolated hearts subjected to salidroside were significantly elevated, while the levels of p-NF-κBp65, p-IκBα, p-IKKα and p-IKKβ were dramatically reduced by salidroside.
Conclusions: The present study comprehensively elaborated the protective effects of salidroside on myocardial injury and demonstrated that AMPK/PGC-1α and AMPK/NF-κB signaling cascades were implicated in the myocardial ischemia–reperfusion injury (I/R) model.

1. Introduction

Cardiovascular disease, a major health problem with complex pathogenesis, is considered as one of the most burdensome diseases of society [36]. In clinical, myocardial ischemia is defined as interrupted blood supply to the left ventricle (LV), which is attributed to the blockade of the coronary artery [17]. It is widely accepted that coronary artery ischemia/reperfusion (I/R) injury occurs during the restoration of coronary blood flow posterior a period of myocardial ischemia and triggers further myocardial injury [8]. However, the development of heart physiology is restricted until the emergence of heart perfusion system. Langendorff heart perfusion system facilitates researchers to explore the relationship between cardiac mechanical activity and oxidative metabolism, thereby it has been widely used in cardiac protection studies rapidly [22]. Experimental heart failure induced by surgical ligation of the left coronary artery in rats is a commonly used model. It is an effective model to mimic myocardial ischemia in rats in spite of a high mortality rate approximately to 50% [11,23]. Besides, it is also convenient to conduct reperfusion. It is commonly believed that a number of factors including oxygen radicals, calcium overload, energy insufficient and inflammation are responsible for pathological process of myocardial I/R injury. However, there is no single hypothesis that can comprehensively explain myocardial I/R injury.

Traditional Chinese medicine has been extensively used by one-fifth of the world's population for centuries and is still acknowledged as an importance source of medicine [4,15]. Salidroside (p-hydroxyphenethyl-b-D-glucoside, structure shown in Fig. 1) is one of the compounds with a biological activity of *Rhodiola rosea L.* which was reported to improve cognitive function, reduce free radical damage, relieve myocardial ischemia, enhance learning and memory, and prevent and treat mountain sickness [26,9]. Furthermore, salidroside is documented to possess

Abbreviations: NF-κB, Nuclear factor-κB; AMPK, AMP-activated protein kinase; IL-6, Interleukin (IL)-6; TNF-α, tumor necrosis factor; ELISA, enzyme-linked immuno sorbent assay; CK, creatine kinase; LDH, lactate dehydrogenase; TTC, 2,3,5-triphenyltetrazoliumchloride.

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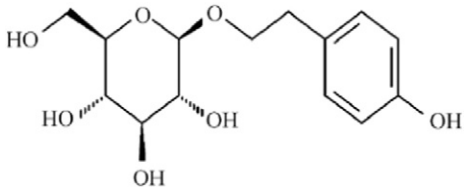

Fig. 1. Structure of salidroside (Sal).

various pharmacological activities, such as anti-inflammatory [5], antioxidant [5], anti-hypoxia [37], neuroprotective [12], hepatoprotective [31] and cardioprotective effects [36]. However, the function of salidroside in coronary artery occlusion-induced myocardial ischemia–reperfusion injury and Langendorff-perfused rat hearts has not been reported until now.

More importantly, there is a notion that energy metabolic imbalance is an important characteristic in no matter coronary artery occlusion-induced rat *in vivo* or Langendorff-perfused isolated rat hearts. AMP-activated protein kinase (AMPK) emerges as a potential therapeutic target for the treatment of diabetes, cancer and cardiovascular disease [18]. It is a stress signaling enzyme regulating energy generation and consumption. Intrinsic AMPK activation and pharmacologic AMPK stimulation protect the heart against ischemic and ischemia–reperfusion injury [21,32], but whether salidroside acts on AMPK site is still known. In addition, whether extrinsic AMPK activation is effective for isolated heart injury has not yet been studied. Emerging evidence suggested that NF-κB is a central transcriptional factor modulating inflammatory mediators. Activation of AMPK has been demonstrated to possess an anti-inflammatory property, which is associated with diminished NF-κB [27]. Thus, the current study assessed the function of AMPK *in vivo* and *in ex vivo*.

2. Materials and methods

2.1. Main reagents and kits

Salidroside was provided by The Second Military Medical University (Shanghai, P.R. China; purity ≥ 99%); Diltiazem was purchased from Simcare Drug Store (Nanjing, P.R. China). TTC was purchased from Sigma–Aldrich (St. Louis, USA). Interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α enzyme-linked immuno sorbent assay (ELISA) kits were supplied by Jiancheng Bioengineering Institute (Nanjing, China), as well as creatine kinase (CK), lactate dehydrogenase (LDH), ATP and glycogen commercial kits. Primary antibodies against p-AMPK, AMPK, PPAR-α, PGC-1α, IκBα, p-IκBα, NF-κBp65, p-NF-κBp65, IKKα, p-IKKα, IKKβ and p-IKKβ antibodies were produced by Cell Signaling Technology (Danvers, USA).

All other chemicals and reagents used for study were of analytical grade and were purchased from approved organizations.

2.2. Animals

Sprague–Dawley (SD) rats (250–300 g) were supplied by Comparative Medicine Centre of Yangzhou University. Rats were maintained on a regular 12 h light/12 h dark cycle at a constant temperature of 22 ± 1 °C and humidity of 40–70%. Standard food and water were provided *ad libitum*.

Ethical standards statement: All animal operations were approved by China Pharmaceutical University (CPU.2012–003).

2.3. Surgical ligation of the left coronary artery and experimental protocol

The surgical protocol was carried out as previously described by Pfeffer et al. [23] with minor modifications. Primarily, rats were anesthetized and restrained. Trachea was incised and catgut was placed surrounding the wound for later use. Then, an incision was made on the skin at the position of the heart. The underlying ribs were exposed by blunt-dissection to provide convenience for heart operation followed. After that, left anterior descending coronary were blocked with special suture. Meanwhile, a positive pressure respirator was responsible for the respiration of experimental rats. An ST-segment elevation was considered as the sign of a successfully developed model. A recovered strong and regular heart rhythm indicated that thoracic cavity could be sutured. The suture of rats in the sham group was not tied. Taking accidental deaths caused by failed surgery into consideration, the number of rat in each group was as follows: sham group, I/R group (model group), I/R + Dil (10 mg/kg) group, I/R + Sal (20 mg/kg) group and IR + Sal (40 mg/kg) group. Sal and Dil were treated for 5 days after surgery.

Rats were anesthetized again 24 h after the operation. Blood samples were collected from carotid artery and centrifuged at 3500 g for 15 min, and then the supernatant were stored at −80 °C for biochemical indicators analysis. Subsequently, rats were sacrificed and hearts were collected for TTC staining, western blot and pathology analysis.

2.4. Electrocardiograms (ECG) measurement

ECG monitored ST-segment alterations along with the whole surgery using the BL-420S Biologic Function Experiment system (Chengdu, China). Furthermore, ST-segment elevation was recorded 24 h after surgery.

2.5. Measurement of pro-inflammatory cytokines in serum

The contents of serum IL-6, IL-1β and TNF-α were detected using ELISA kits according to the manufacturer's instructions.

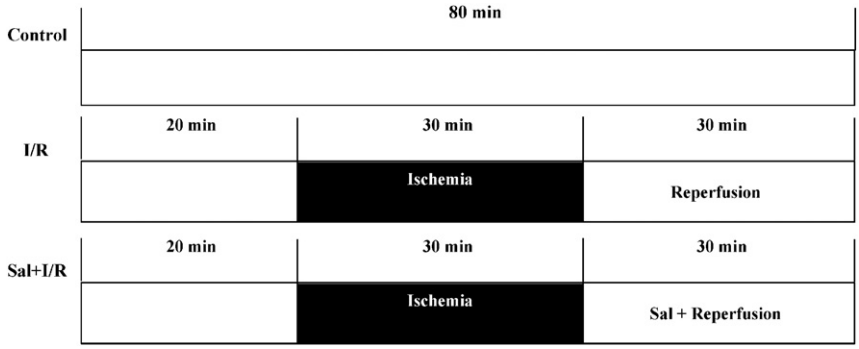

Fig. 2. Experimental protocols *in ex vivo*. Control group hearts were perfused for the 80 min stabilization period. I/R group hearts were stabilized for 20 min, and then global ischemia (no flow) were conduct for 30 min. Finally, reperfusion was subjected to them for 30 min with K-H buffer. Hearts in drug-administrated group were treated in line with the described procedures, whereas reperfusion was subjected to them with Sal-containing or Dil-containing K-H buffer for 30 min.