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Protection of Water Extract from *Paeoniae Radix Rubra* against Myocardial Ischemia in Mice Induced by Isoproterenol

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Abstract: **Objective** To study the protective effect of the water extract from *Paeoniae Radix Rubra* (WEPRR) against myocardial ischemia in mice induced by isoproterenol (ISO). **Methods** The mice were randomly divided into six groups: positive control, normal control, model, low-, mid-, and high-dose [0.3, 0.6, and 1.2 g/(kg·d)] WEPRR groups. The mice in WEPRR groups were ig administered with WEPRR, the mice in the positive control group were ig administered with 0.98% Di'ao Xinxue Kang [(0.195 g/(kg·d)], and the mice in normal and model groups were ig administered with the same volume of physiological saline once daily for consecutive 11 d. On the day 7 from the beginning of the ig administration, the mice in the model and WEPRR groups were ip perfused with 0.02 g/(kg·d) ISO. After 15 min of the last medication, the mice were anesthetized with isoflurane gas, the blood was collected through venous sinus of eye orbit, then the mice were killed. The heart tissues were rapidly removed from the mice, washed in physiological saline, soaked in filter paper, and stored in −80 ℃ until use. The activities of lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), creatine kinase (CK) in serum, superoxide dismutase (SOD), and the contents of malondialdehyde (MDA) in the heart of mice were determined, respectively. **Results** Compared with the model group, the activities of LDH, CK-MB, and CK in serum, and the SOD of mice in the positive control and WEPRR groups were increased and the content of MDA in heart was decreased. **Conclusion** The WEPRR has the cardioprotective activities on ISO-induced myocardial ischemia.

Key words: acute myocardial ischemic; cardioprotective activities; isoproterenol; lactate dehydrogenase; *Paeoniae Radix Rubra*
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Introduction

There are two species under the monograph of *Paeoniae Rubrae Radix* (PRR, *Chishao* in Chinese) in *Pharmacopoeia of People's Republic of China 2010*, *Paeonia lactiflora* Pall. and *Paeonia veitchii* Lynch (Xu *et al*, 2009). PRR is a traditional Chinese medicinal material for treating some diseases including diabetes, artherosclerosis, dementia, etc (Su *et al*, 2010; Zhang *et al*, 2007, 2009; Liu *et al*, 2005). There are enough evidences for oxidative stress involvement in these related diseases. Oxidative stress is characterized by the overproduction of reactive oxygen species (ROS) (Cesare *et al*, 2007) which are chemically unstable and highly reactive and are able to cause cellular and tissue damage when their generation exceeds the endogenous ability to destroy them (Zhang *et al*, 2007). The recent evidence suggests that PRR could be used as natural excellent antibiotic and anti-oxidative material (Shang *et al*, 2007; Du *et al*, 2010).

The heart is one of the major organs affected by ROS. The model of isoproterenol (ISO)-induced acute myocardial ischemia (AMI) is considered as one of the most widely used experimental model to study the beneficial effects of many drugs and cardiac function (Grimm, Elsner, and Schunkert, 1998). The model has been reported to show many metabolic and morphologic aberrations in heart tissue of the experimental animals similar to those observed in human myocardial ischemia (Aman, Hardik, and Balaraman, 2011; Nirmala and Puvanakrishnan, 1996; Wexler and Greenberg, 1978).

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Several mechanisms of ISO-induced AMI have been suggested including oxidative stress which is one of the main mechanisms more probably (Senthil, Sridevi, and Pugalendi, 2007). In the present study, the protective effects of PRR on ISO-induced AMI in mice are investigated.

Materials and methods

Materials

Paeoniae Radix Rubra was purchased from Zhangyuan Pharmaceutical Co., Ltd., China; the isoprenalineride hydrochloride injection was produced from Shanghai Harvest Pharmaceutical Co., Ltd., China

Extract of PRR

PRR was extracted with distilled water. And then the extracted solution was filtered by using a clean cloth. The extract was concentrated to about 1 g crude drug per milliliter in rotary vacuum evaporator. Paeoniflorin is one of the main components in PRR, and the qualitative and quantitative analyses were performed by HPLC coupled with ultraviolet in *Pharmacopoeia of People's Republic of China 2010*. The stock solution contained 60 mg/mL paeoniflorin in water. The concentrated extract was kept in 4 ℃ for use.

Treatment of animals

Healthy adult male Kunming mice of clean degree, weighing approximately 22—30 g, were used for the experiment. The animals were housed individually in polypropylene cages under hygienic conditions and maintained at room temperature. They all received pellet diet and water *ad libitum*.

Mice were randomly divided into six groups: positive control, normal control, model, and three water extract from PRR (WEPRR) groups. Mice in WEPRR groups were ig administered with WEPRR [0.3, 0.6, and 1.2 g/(kg·d)]. Mice in positive control group were ig administered with 0.98% Di'ao Xinxue Kang [DXK, (0.195 g/(kg·d), batch No. 1211027] produced by Chengdu Di'ao Drug-making Co., Ltd. Mice in normal and model groups were ig administered with the same volume of physiological saline once daily for consecutive 11 d. On the day 7 from the beginning of the ig administration, mice were ip perfused with ISO [0.02 g/(kg·d)] in the model and WEPRR groups. Fifteen minutes after the last medication, the mice were treated with isoflurane gas anesthesia, and blood was collected through mice venous sinus of eye orbit, then the heart tissue was rapidly removed from the mice, washed in physiological saline, soaked in filter paper, and stored frozen (−80 ℃) until analysis.

Biochemical analysis

The activities of lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), creatine kinase (CK) in serum and superoxide dismutase (SOD) and the content of malondialdehyde (MDA) in the heart of mice were determined, respectively, according to the protocol of assay kits from Nanjing Jiancheng Biotechnology Ltd., Inc. (Nanjing, China).

Statistical analysis

Results are expressed as $\bar{x} \pm s$. The data were statistically analyzed by One-way analysis of variance (ANOVA) using SPSS 10.0, the statistical significance was $P < 0.05$.

Results

Effects of WEPRR on CK, CK-MB, and LDH levels in serum

The effects of WEPRR on CK, CK-MB, and LDH levels in serum were shown in Fig. 1. The results

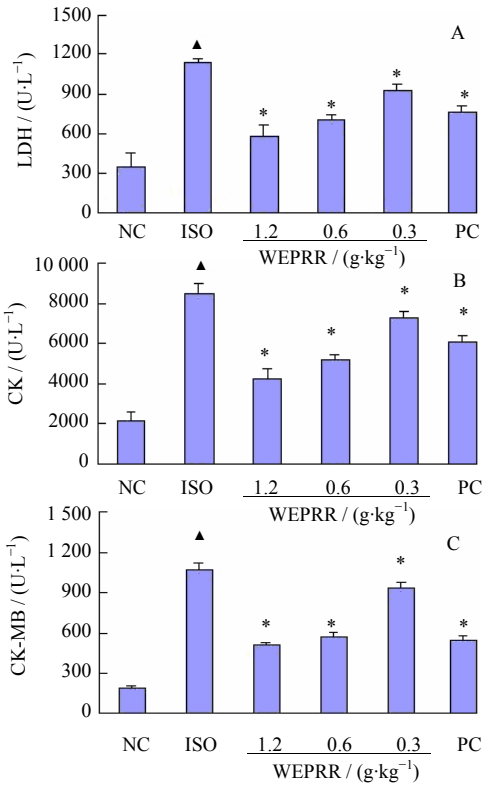

Fig. 1 Effects of WEPRR on activities of LDH (A), CK (B), and CK-MB (C) in serum ($\bar{x} \pm s$, $n = 9$)

▲$P < 0.01$ *vs* normal control group; *$P < 0.05$ *vs* ISO group, same as below

indicated that the levels of CK, CK-MB, and LDH activities in serum of ISO-induced AMI group were significantly elevated in contrast with the normal control group ($P < 0.01$). Pretreatment with DXK and WEPRR at three different doses improved the activities of LDH, CK, and CK-MB significantly compared with ISO-treated group ($P < 0.05$).

Effects of WEPRR on MDA level and SOD activities

The effects of WEPRR on MDA level and SOD activities in the heart tissue were shown in Fig. 2. The results showed that the MDA level in the heart tissue of mice in the model group was significantly elevated in contrast with that in the normal control group. Pretreatment with WEPRR (drug group) and DXK (positive group) decreased the elevated heart MDA level; While, the SOD activity in the heart tissue of mice in the model group lowered significantly in contrast with that in the normal control group. Pretreatment with WEPRR and DXK increased the lowered SOD activity in heart tissue of mice in contrast with that in the model group.

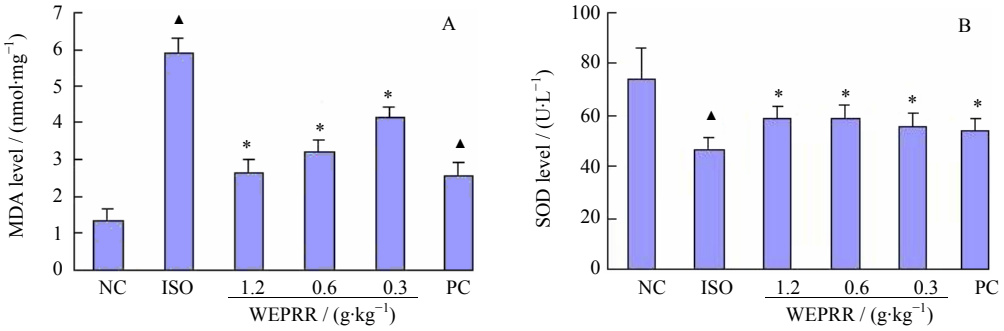

Fig. 2 Effects of WEPRR on content of MDA (A) and activity of SOD (B) ($\bar{x} \pm s$, $n = 9$)

Discussion

ISO is a synthetic catecholamine that could induce myocardial ischemia due to producing excessive free radicals resulting from oxidative metabolism of catecholamines (Senthil, Chandramohan, and Pugalendi, 2007). WEPRR pretreatment could prevent the elevation of CK, CK-MB, LDH, MDA and decrease the activities of the enzyme SOD. The oxidative stress may be exerted through the quinone metabolites of ISO, which reacts with oxygen to produce ROS (Rathore *et al*, 1998a), and myocardial cells are damaged or destroyed due to deficient oxygen supply and the cell membrane becomes permeable or may rupture (Senthil, Sridevi, and Pugalendi, 2007), which results in the leakage of enzymes, including CK, CK-MB, and LDH. This accounts for the increased activities of these enzymes in serum of mice with myocardial ischemia induced by ISO as compared to the normal control group.

One of the major causes of ischemic heart disease is an imbalance between oxidants and anti-oxidant defense, and interferene with SOD (Senthil, Chandramohan, and Pugalendi, 2007; Rathore *et al*, 1998a; 1998b). This might help to explain the decreased activity of SOD in myocardium tissue.

Lipid metabolism plays an important role in AMI (Mathew, Menon, and Kurup, 1981), and MDA production is recognized as a marker for lipid peroxide and as an end-product of peroxidation (Slater, 1984; Seung and Kyung, 2003). The present study indicates that WEPRR pretreatment could prevent the elevation of MDA in heart-muscle tissue of mice with myocardial ischemia induced by ISO.

In conclusion, it is evident from the present study that WEPRP has a remarkerable protection against ISO-induced AMI.

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