Protective effect of the extract of Yi-Qi-Fu-Mai preparation on hypoxia-induced heart injury in mice

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[ABSTRACT] Yi-Qi-Fu-Mai (YQFM) is extensively used clinically to treat cardiovascular diseases in China. To explore the anti-hypoxia effect of the extract of YQFM preparation (EYQFM), the EYQFM (1.4, 2.8, and 5.5 g·kg⁻¹·d⁻¹) was assessed for its heart-protective effect in a chronic intermittent hypoxia (CIH) animal model (oxygen pressure 7%–8%, 20 min per day) for 28 days of treatment. Betaloc (0.151 6 g·kg⁻¹·d⁻¹) was used as a positive control. The histopathological analyses of heart in CIH mice were conducted. Several cardiac state parameters, such as left ventricular ejection fractions (EF), stroke volume (SV), expression of creatine kinase (CK), lactate dehydrogenase (LDH), superoxide dismutase (SOD), and malondialdehyde (MDA) were measured. The results showed that treatment with EYQFM markedly reversed swelling of the endothelial cells and vacuolization in the heart when compared with the model group. Further study demonstrated that EYQFM significantly improved ventricular myocardial contractility by increasing EF and SV. In addition, EYQFM inhibited the activity of CK, LDH, decreased the level of MDA and improved SOD activity. The results demonstrated that EYQFM significantly improved the tolerability of myocardium to hypoxia and ameliorated the cardiac damage in the CIH model.

(KEY WORDS) Extract of YQFM preparation; Chronic intermittent hypoxia; Cardiac damage


Introduction

Several traditional Chinese medicines have shown therapeutic effects against heart diseases because of their holistic and multi-target pharmacological actions [1-2]. Yi-Qi-Fu-Mai (YQFM), a modified preparation of the classic prescription Shengmai San (SMS), has been used to treat the deficiency of both Qi and Yin syndrome (DQYS), according to the theory of traditional Chinese medicine (TCM). Moreover, YQFM has already been used in clinical practice in China to prevent cardiovascular diseases, such as coronary heart disease.

Compared with admixture decoction of SMS, YQFM is a mixture of the extracts from three crude herbs: Radix Ginseng, Radix Ophiopogonis, and Fructus Schisandrae, using different extraction process; it removes most of large molecules of SMS. This simplified prescription is much easier for quality control, keeping the major therapeutic efficacy of the primary prescription.

Since oxygen has been proven to be crucial in the pathogenesis of myocardial disease [4-5], the possible effects of YQFM on oxygen have attracted more attention from researchers. Previous reports have mainly explored the antioxidant capacity of SMS to protect against post-ischemic myocardial dysfunction [6-8]. In addition, the effects of SMS have been mostly investigated using isoproterenol-induced myocardial injury models or myocardial ischemia-reperfusion caused by coronary arteries ligation [9-10]. However, the anti-hypoxic effect of SMS or YQFM on myocardial protection has not been taken into account. In order to
investigate the heart injury induced by hypoxia, chronic intermittent hypoxia (CIH) model was selected in the present study. CIH has been used to study obstructive sleep apnea (OSA) that can produce many abnormalities such as the abnormal lipid biosynthesis [11-12], atherosclerosis [13], and liver injury [14]. Our previous study has analyzed macroscopic symptoms of DQYS [15]. However, the relationship between behavior. It has been found that the CIH model mimics the signs of the CIH mice, such as body weight, food intake, and behavior. It has been found that the CIH model mimics the symptoms of DQYS [15]. However, the relationship between CIH and heart injury has not been fully investigated. The extract of YQFM preparation (EYQFM), (Tasly Pharmaceuticals Company) possesses the same efficacy as YQFM preparation (EYQFM). In addition, EYQFM is convenient for further research of YQFM. We therefore investigated the protective effects of the EYQFM against the heart injury induced by hypoxia in the CIH model in the present study. The pathological changes of heart, the function of left ventricular as well as the activities of related serum enzymes in CIH mice were evaluated. The results demonstrated that EYQFM can significantly improve myocardial injury in the CIH mice.

Materials and Methods

Reagents and chemicals

Lactate dehydrogenase (LDH), creatine kinase (CK), superoxide dismutase (SOD), and malondialdehyde (MDA) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The EYQFM was a kindly gift from Tasly Pharmaceuticals Company (Tianjin, China).

Experimental animals

Seventy-two male Institute of Cancer Research (ICR) mice weighing 18 to 22 g were provided by the Experimental Animal Center of Yangzhou University, Yangzhou, China. The mice were housed in air conditioned rooms with controlled temperature (22 ± 2 °C), ambient humidity (50% ± 10%), and 12 h/12 h light/dark cycle, with free access to food and water. All procedures were operated in accordance with the National Institute of Health’s guidelines. All the animal experiments were approved by the Animal Ethics Committee of School of Chinese Materia Medica, China Pharmaceutical University, Nanjing, China.

Chronic intermittent hypoxia (CIH) mouse model

The mice were randomized into six groups of twelve mice each: control group, hypoxia model group, betaloc group, and three EYQFM groups treated with different dosages. The low, medium and high dosages of EYQFM were 1.4, 2.8, and 5.5 g·kg⁻¹·d⁻¹, (termed to l-EYQFM, m-EYQFM and h-EYQFM groups, respectively). The betaloc group and the three EYQFM groups mice were given intragastric administration of betaloc (0.1516 g·kg⁻¹·d⁻¹) and the EYQFM (1.4, 2.8, and 5.5 g·kg⁻¹·d⁻¹), respectively, while the control group and hypoxia model group were given double-distilled water once a day by gavage. After treatment, all the mice, except for the control group, were placed into hypoxic box with oxygen pressure 7%–8% for 20 min once a day for 28 consecutive days to produce the chronic myocardial injury.

Examination of histopathology

The heart tissue of the mice were fixed with formalin and embedded with paraffin, then sliced to 5 μm sections. After hematoxylin-eosin staining, the sections were mounted and the pictures were taken under a light microscope (OLYMPUS DX45, Japan).

Acquisition of left ventricular ejection fractions and stroke volume

Ultrasonic images were recorded on the 28th day after intraperitoneal injection of 4% chloral hydrate (0.1 mL/10 g). The left ventricular ejection fractions and stroke volume were calculated based on the ultrasonic images.

Evaluation of LDH, CK, SOD activities and MDA levels

After collection of the blood from the orbits of the mice, the supernatant was gathered by centrifugation and subjected to examine biochemical indicators using assay kits, following the manufacturers’ instructions.

Statistical analysis

The data are presented by mean ± SD. Differences between the groups were analyzed by one-way ANOVA followed by either Dunnett’s posthoc test or student t test using GraphPad Prism 5.0 software (GraphPad Software, Inc., California, United States). P < 0.05 was considered statistically significant.

Results

Effects of EYQFM on the cardiac pathological changes

The control mice showed no obvious pathological damages in myocardial tissues (Figs. A1 and A2). The CIH model group showed obvious swelling of the endothelial cells (Fig. B1) and cell vacuolar degeneration (Fig. B2) in the cardiac tissues. EYQFM and betaloc significantly alleviated the pathological damages in the CIH mice. The mice treated with EYQFM and betaloc showed less swelling of endothelial cells (Figs. D1, E1, F1, and C1) and cell vacuolar degeneration (Figs. D2, E2, F2, and C2), compared with the CIH model control mice.

Effects of EYQFM on the stroke volume (SV) and left ventricular ejection fractions (EF)

As shown in Fig. 2, the SV and EF values of the hypoxia model group were significantly reduced, compared with that of the control group (P < 0.01). The mice treated with m-EYQFM, h-EYQFM and betaloc showed much higher SV values than the model group (P < 0.05). Similarly, 1-EYQFM and m-EYQFM treatment significantly elevated the EF (P < 0.05). High dosage of EYQFM produced an increase in the EF which was equal to the response of betaloc.

Effects of EYQFM on the activity of LDH and CK

Compared with the normal mice, the hypoxia model mice produced significant increases in the CK activity (P < 0.01) and LDH activity (P < 0.05). Similar to the positive control, treatments with all three dosages of EYQFM produced significant decreases on the CK activity (P < 0.05, l-EYQFM, m-EYQFM vs control; P < 0.01, h-EYQFM vs control).
Fig. 1 Effects of EYQFM on pathological changes in the CIH mice (A- F) Hematoxylin - eosin staining; Bar: 20 µm; magnification × 400; normal control group (A1, A2), hypoxia-induced injury model group (B1, B2), betaloc group (C1, C2), l-EYQFM group (D1, D2), m-EYQFM group (E1, E2), h-EYQFM group (F1, F2). Swelling of the endothelial cells in cardiac tissue (A1-F1), vacuolization in cardiac tissue (A2-F2).

Treatment with m-EYQFM, h-EYQFM and betaloc also produced significantly decreases in the LDH efflux ($P < 0.05$).

**Effects of EYQFM on oxidative injury indexes**

The CIH mice displayed a higher MDA level ($P < 0.05$) but decreased SOD activity ($P < 0.05$) than the normal controls. Compared with the model group, the MDA level significantly declined significantly in the mice treated with m-EYQFM, h-EYQFM, and betaloc (all $P < 0.05$). The activities of SOD in all the EYQFM treated groups and betaloc group increased significantly, compared with that of the model group ($P < 0.05$).

**Discussion**

Heart is the one of the biggest consumers of oxygen and vulnerable organs in human body $^{[17]}$ and sufficient oxygen supply is critical to the normal function of heart $^{[18-21]}$. In the present study, the CIH model was used to investigate the
Fig. 2 Effects of EYQFM on EF and SV in the CIH mice (mean ± SEM, n = 7) (A) Left ventricular ejection fractions of all groups. (B) Stroke volume of all groups. \(^*\)P < 0.05, \(^{##}\)P < 0.01 vs control mice; \(^\dagger\)P < 0.05, \(^{\ddagger\ddagger}\)P < 0.01 vs CIH mice.

Fig. 3 Effects of EYQFM on CK and LDH activity in the CIH mice (mean ± SEM, n = 12) (A) CK activity of all groups. (B) LDH activity of all groups. \(^\#\)P < 0.05, \(^{##}\)P < 0.01 vs control mice; \(^\dagger\)P < 0.05, \(^{\ddagger\ddagger}\)P < 0.01 vs CIH mice.

Fig. 4 Effects of EYQFM on MDA level and SOD activity in the CIH mice (mean ± SEM, n = 12) (A) MDA level of all groups. (B) SOD activity of all groups. \(^\#\)P < 0.05, \(^{##}\)P < 0.01 vs control mice; \(^\dagger\)P < 0.05, \(^{\ddagger\ddagger}\)P < 0.01 vs CIH mice.

The protective effects of EYQFM on hypoxia-induced heart injury in mice. The pathologic examination can visually present the heart injury conditions. Our results indicated that the EYQFM reversed the hypoxia heart damages, including swelling of the endothelial cells and cell vacuolar degeneration. According to these results, EYQFM could significantly reduce pathological damage on heart induced by CIH.

As an important part of heart, the ventricles are main energy source of heart, especially left ventricular, which takes the important mission of the blood supply to the body. At present, the main approach to assessing the contractility of left ventricular is the echocardiography for its noninvasive and simple operation. Furthermore, the most common clinical parameters used to evaluate the function of left ventricular are the EF and SV. In the present study, the
values of EF and SV were used to evaluate the heart function state. Compared with control group, the values of EF and SV in CIH mice decreased significantly ($P < 0.01$), indicating that CIH can induce cardiac systolic dysfunction. The EYQFM was demonstrated to be useful in improving cardiac function of the mice under chronic hypoxia condition by increasing EF and SV of the left ventricular.

Additionally, the activities of some cardiac marker enzymes can reflect the pathological process of myocardial diseases. After the myocardial damage, the CK and LDH can leak out of the myocardial cells thus the increase of the activity of CK and LDH is also known as the signal of heart damage. Therefore, the measurement of CK and LDH is used as a sensitive index of diagnosing heart disease in the clinic, especially the activity of CK which are considered more reliable than electrocardiogram in diagnosis of myocardial infarction. The results from the present study supported the hypothesis that the CIH would induce the leakage of CK and LDH. Moreover, the EYQFM decreased the activity of CK and LDH by mitigating the heart damage.

Previous reports have shown that SOD is an essential antioxidant enzyme to scavenge reactive oxygen radicals [24-25]. In those normal control mice, SOD can protect heart from oxidative injury by detoxifying the superoxide anion to hydrogen peroxide [26]. In CIH mice, the decreasing activity of SOD can cause assault to the heart by the oxygen radicals. Furthermore, EYQFM enhanced the activity of SOD to antioxidative injury by the oxygen radicals. In vivo, the EYQFM increased the activity of SOD and antioxidant enzyme to scavenge reactive oxygen radicals [24-25].

Conclusion

Our investigation and some previous studies have shown that the CIH model can result in significant heart injury. EYQFM acted as an effective anti-hypoxic agent by ameliorating the pathological damage of heart, reducing the activity of CK and LDH, decreasing the level of MDA, as well as increasing EF, SV, and SOD activity. The underlying anti-hypoxia mechanisms research of EYQFM will be studied in the future.

References


