The modified Yi qi decoction protects cardiac ischemia-reperfusion induced injury in rats

Xiao Yu†, Xiao-Dong Zhao†, Rong-Qi Bao, Jia-Yu Yu, Guo-Xing Zhang and Jing-Wei Chen

Abstract

Background: To investigate the effects and involved mechanisms of the modified Yi Qi decoction (MYQ) in cardiac ischemia-reperfusion (IR) induced injury.

Methods: Male Sprague-Dawley rats were subjected to a 30-min coronary arterial occlusion followed by reperfusion, low or high dose decoction of MYQ was administrated orally for 1 week or 1 month.

Results: Both in 1 week and 1 month IR rat groups, cardiac function indexes were significantly impaired compared with sham group rats, accompanied with higher ratio of infarct size to risk size, decreased expressions of sodium calcium exchanger (NCX1) and sarcoplasmic reticulum Ca²⁺-ATPase (Serca2a), and different expressions of autophagic proteins, Beclin-1 and LC3. Treatment with MYQ (low or high dose) for 1 week showed no marked beneficial effects on cardiac function and cardiac injury (ratio of infarct size to risk size), although expressions of anti-apoptotic protein, Bcl-2, NCX1 and Serca2a were increased. Treatment with MYQ (low or high dose) for 1 month showed significantly improved effects on cardiac function and cardiac injury (ratio of infarct size to risk size), accompanied with increase of Bcl-2, NCX1 and Serca2a expressions, and decrease of Bax (a pro-apoptotic protein) and Beclin-1 expressions.

Conclusions: The results show that MYQ have potential therapeutic effects on IR-induced cardiac injury, which may be through regulation of apoptotic proteins, cytosolic Ca²⁺ handling proteins and autophagic proteins signal pathways.

Keywords: Modified Yi qi decoction (MYQ), Cardiac ischemia-reperfusion, Apoptosis, Calcium handling proteins, Autophagy

Background

Heart failure is still a major public health problem worldwide with high mortality and morbidity [1, 2]. It can be the result of various diseases, coronary artery disease and subsequent myocardial infarction is a common cause of heart failure. The rapid energy depletion suppresses metabolic activity and leads to the induction of cardiomyocytes cell death pathways. Reperfusion ameliorates the extent of cell death, but in turn it invokes lethal reperfusion injury, which may proceed via necrosis, apoptosis and autophagy [3]. The mechanisms responsible for ischemia-reperfusion (I/R) injury have been widely investigated, calcium overload, excessive production of reactive oxygen species (ROS), and the release of inflammatory factors are the major causative factors of cardiac I/R injury [4–6]. A large number of strategies has been proposed to improve cardiac function related to those mechanisms, however, the clinic outcomes are still not satisfied.

Chinese herbal medicine, especially combined herbal formulation, has been widely applied for cardiovascular diseases for hundreds of years. Possible mechanisms have been well reviewed [7], including: (1) anti-oxidation, (2)
anti-inflammation, (3) anti-apoptosis, (4) protection of mitochondria function, (5) promoting angiogenesis, (6) increasing bone marrow derived stem cells migration, (7) inhibiting Ca$^{2+}$ overload. According to the theory of Traditional Chinese Medicine, the principal mechanism of cardiovascular disease is considered to be a disorder or deficiency of Qi (energy) and a disorder of the circulation (blood stasis) which lead to severe pain and even death. Therefore, the main aims of Chinese herbs and herbal formulations in cardiovascular diseases treatment are to regulate or replenish Qi, and/or to unblock circulation or resolve blood stasis. However, studies of Chinese decoctions or formulations in treatment of cardiovascular diseases are relatively scarce, although decoction and formulations are the main forms of therapy in Traditional Chinese Medicine practice.

Wu Meng Therapy has long history for more than 2500 years. Xi Feng-Lin, a grandmaster of Chinese Medicine, established ten principles for treatment of coronary disease according to Synopsis of Preions of the Golden Chamber written by Zhang Zhong-Jing of Eastern Han Dynasty (150–219), its main concept is treatment of heart diseases should combine with improvement of heart, spleen and stomach functions. The modified Yi Qi decoction (MYQ) consists of five herbs including Milkvetch Root, Semen Pharbitidis, Cinnamom Twig, Fructus Amomi, and White Peony Root. Milkvetch Root, has been used for more over 2000 years in China, can strengthen immune function, protect liver, promote urination, resist aging and stress, reduce blood pressure and extensively resist bacterium [8–12]. Semen Pharbitidis has anti-tumor and anti-metastasis effects in Lewis lung cancer [13]. Recently, it was also reported that a Traditional Chinese Medicine Herbal Ointment, mainly containing Semen Pharbitidis could reduce malignant pleural effusion [14]. Cinnamom Twig also has been widely used in traditional Chinese medicine for the treatment of endometriosis-related symptomatic discomfort [15], uterine fibroid [16]. Recently, anti-hyperglycemic and antioxidant activities of twig extract from Cinnamomum osmophloeum also have been reported [17]. Fructus Amomi also has hypoglycemic, antioxidative and anti-allergic inflammatory activities [18–20]. White Peony Root is a component herb of many traditional formulae, such as Siwu-Tang, which has been widely used in treating palpitation, dysmenorrhea, chronic inflammation, anemia, depression, diabetic peripheral neuropathic pain and acute radiation-induced esophagitis [21–24].

Importantly, Yi Qi decoction has been widely applied in ancient Chinese patients and recent scientific researches supporting its beneficial effects. Yin et al. demonstrated that a Yi Qi decoction, Shu-Mai-Tang could attenuate TNFα-induced myocardial fibrosis in myocardial ischemia rats [25]. Mark et al. also reported that another Yi Qi decoction, Dang-Gui Buxue Tang, possesses a more potent cardioprotective effect than its extracts from different component herbs and enhances glutathione status in rat heart mitochondria and erythrocytes [26]. Other Yi Qi decoctions, Shu-mai-tang, Dan-Chuan-Hong also have beneficial effects on angiogenesis, arteriogenesis, anti-apoptosis and improvement of cardiac function in rats with myocardial ischemia [27, 28]. Recently, Danshen-Gegen decoction, also belonging to Yi Qi decoction, has been reported to protect the myocardium against I/R injury via the redox-sensitive PKCe/mKATP pathway in rats [29], and protect against hypoxia/reoxygenation-induced apoptosis by inhibiting mitochondrial permeability transition via the redox-sensitive ERK/Nrf2 and PKCe/mKATP pathways in H9c2 cardiomyocytes [30]. These above-mentioned litterateurs suggest that Yi Qi decoction may protect cardiac against I/R injury, however, different compositions of decoction may have different effects and via different signal pathways. Therefore, in the present study we carry out our investigation into the effects and mechanisms of Yi Qi decoction with modification (MYQ) on cardiac I/R induced injury.

In the present study, we applied rat I/R model and treated with different dosages of MYQ after reperfusion for 1 week or 1 month. Cardiac performance after one-week and one-month reperfusion is examined to testify the therapeutic effects of MYQ. Furthermore, mechanisms of therapeutic effects of MYQ are investigated by detecting the expressions of apoptotic signal pathway proteins, calcium handling proteins, autophagic signal pathway proteins.

Methods
Preparation of MYQ
MYQ was prepared from five dried raw materials (Table 1) purchased from Suzhou Chinese Traditional Medicine Hospital (Suzhou, China) and authenticated by a pharmacist of traditional Chinese medicine in Suzhou Chinese Traditional Medicine Hospital and all voucher specimens are deposited in the herbarium center of Suzhou Chinese Traditional Medicine Hospital. All raw materials were extracted by boiling in distilled water (about 6-fold the weight of the mixture) at 100 °C for 20 min and then filtered. The filtrates were stored at −80 °C for further application.

Experimental animals
Ten-week-old male Sprague-Dawley rats were purchased from Shanghai Laboratory Animal Center. Rats were housed under optimal conditions with standard hygiene, kept at a temperature of 25 °C with a 12/12 light/dark cycle, fed with standard rat chow and water ad libitum. The experiments were performed in according with the
National Institutes of Health Guidelines for the Use of Laboratory Animals (NIH, publication number 85–23, revised 1996.), which were approved by and performed according to guidelines for the care and use of animals established by Soochow University of Animal Care and Use Committee. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize animal suffering.

Myocardial I/R model
The I/R model was performed as our previous report [31]. Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), cardiac I/R was performed by exposing the heart at the fifth intercostal space followed by a slipknot (6–0 silk) below the left descending coronary artery. Regional left ventricular ischemia was performed via occlusion of the coronary artery by clamping it together with the propylene tube. After 30 min of ischemia, the slipknot was released and ischemic part was subjected to reperfusion. Two hours later, rats were treated with or without MYQ solution for 1 ml (low dose, equals to clinical patient’s dosage calculated according to body surface area) or 4 ml (high dose) by gastric feeding. Then, everyday rats were treated three times with above-mentioned dosages. Sham group is without occlusion (n = 10), low dose treatment of I/R rats lasts for 1 week (n = 10) or 1 month (n = 10), high dose treatment of I/R rats also lasts for 1 week (n = 9) or 1 month (n = 9).

Cardiac function measurements
One week or 1 month later after I/R, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and hemodynamic parameters were measured using a heart performance analysis system (ALCBIO, Shanghai Alcott Biotech CO., LTD.). The left femoral artery and right common carotid artery were isolated. A polystyrene PE-50 catheter was inserted into the left ventricle via right common carotid artery, with the other end connected to the analysis system. The major parameters of cardiac function were derived or calculated from the continuously obtained pressure signal, including systolic arterial pressure (SAP), the rate of maximum positive and negative left ventricular pressure development (± LVdp/dt max), and the left ventricular end-diastolic pressure (LVEDP), etc.

Measurement of ratio of myocardial infarct area to area at risk
After rat’s cardiac function was measured under anesthetized condition with sodium pentobarbital (50 mg/kg i.p.), rat hearts were excised immediately and perfused with Evans blue (1%, 4 ml) via the coronary artery under ligation of the left descending coronary artery with the remained sutures. Hearts were traverse cut into 1–2 mm slices along the ligation point, placed in 1.25% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma, USA) solution in PBS, incubate for 10 min at 37 °C. The ischemic regions (area at risk, AAR) and the infarct area (white area is not stained by TTC) were recorded with a digital camera, and blue area (stained by Evans blue; non-ischemic area) were analyzed with a digital imaging system (NIH image software). The ratio of myocardial infarct area to area-at-risk (AAR) was calculated.

Western blot analysis
Myocardial tissues (AAR tissue) were homogenized with RIPA buffer (50 mm Tris, ph 7.0, 150 mM NaCl, 1% Triton-X-100) containing phenylmenthanesulfonyl fluoride (R&D Systems Inc., Minneapolis, US). Homogenates were centrifuged at 12,000×g for 10 min at 4 °C. Cell protein were separated by SDS-PAGE and transferred to PVDF membranes (Hybond TM-ECL; Amersham Pharmacia Biotech, Inc.). The membranes were blocked in 5% nonfat milk in PBS and 0.1% Tween-20 at room temperature. The blots were then incubated with primary antibody: anti-Caspase-3 antibody (1:1000, abcam, Inc.), anti-Bcl-2 antibody (1:1000, Immunoway Biotech, Inc.), anti-Bax antibody (1:1000, abcam, Inc.), anti-Beclin-1 (1:1000, Santa Cruz Biotech, Inc.), anti-LC3 (1:1000, abcam, Inc.), anti-NCX1 (1:1000, abcam, Inc.), anti-Serca2a (1:1000, abcam, Inc.) or anti-GAPDH (Santa Cruz Biotech, Inc.). Then the membranes were incubated for 1 h with a secondary antibody (HRP-conjugated anti-rabbit Ig-G, 1:2000). Excess antibody was washed off with TBS-T three times (15 min each) before incubation enhanced chemiluminescent reagent (ECL, R&D Systems Inc., Minneapolis, USA) for 1 min. Subsequently, the membrane was exposed to X-ray film. Immunoreactive bands were detected by the analysis of X-ray films using the software of Image J. The quantity of target proteins is normalized by GAPDH expression.

Table 1 Composition of MYQ

| Chinese name | Latin name | English name | Amount (g) | Place of origin |
|--------------|------------|--------------|------------|-----------------|
| Huang Qi     | Astragalus membranaceus var. mongholicus (Bunge) P.K. Hsiao | Milkvetch Root | 30 | Inner Mongolia, China |
| Hei Chou     | Pharbitis nil (L) Choisy | Semen Pharbitidis | 10 | Jiangsu, China |
| Gui Zhi      | Cinnamomum cassia (L) J. Presl | Cinnamom Twig | 10 | Guangdong, China |
| Sha Ren      | Amomum villosum (L.) Choisy | Fructus Amomi | 5 | Guangdong, China |
| Bai Shao     | Paeonia lactiflora Lour. | White Peony Root | 10 | Zhejian, China |

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Statistical analysis
The SPSS 18.0 software was used for statistical analysis. Data were presented as the mean ± S.E.M. Grouped data were analyzed using a one-way analysis of variance followed by the Student-Newman-Keuls test between each group. A P value <0.05 was considered to be statistically significant.

Results
Effects of MYQ on cardiac function after I/R injury
To determine the effects of MYQ on cardiac function in rat subject to I/R injury, cardiac function measurements were performed one week or one month after reperfusion. I/R significantly decreases cardiac function compared with the sham control group, by decreasing the systolic arterial pressure (SAP), Pmax, ± dp/dtmax, LVEDP and other parameters (Table 2). One-week treatment with MYQ improves the cardiac function parameters under high dosage; One-month treatment with MYQ significantly ameliorates cardiac function both under low and high dosage.

Effects of MYQ on myocardial infarct size after I/R injury
Cardiomyocyte injury is characterized by myocardial infarct size. To determine whether MYQ attenuates I/R-induced cardiomyocyte injury, ratio of infarct size to area-at-risk was calculated. Our data show that ratio of infarct size to area-at risk was still markedly high after one-week or one-month reperfusion. Treatment with MYQ for 1 week did not significantly change the ratio, however, treatment for 1 month significantly reduces the ratio (Fig. 1). Data suggest the long-term therapeutic effects of MYQ on I/R-induced cardiomyocyte injury.

Effects of MYQ on apoptotic signal pathway in rat I/R model
Bcl-2 and Bax genes are reported to play a crucial role in cell survival or death after apoptotic stimuli [32]. Caspase-3 is also an important component of the apoptotic pathway [33]. The effects of MYQ on Bcl-2, Bax, and caspase-3 expression in myocardial tissue were analyzed by western blot. I/R for one-week or one month did not show any marked effects on the expression of these proteins compared with sham group. However, anti-apoptotic protein, Bcl-2 was significantly increases in high doses groups of MYQ treatment for one-week or one-month compared with I/R group (Fig. 2a). In addition, expression of pro-apoptotic protein, Bax, was markedly reduced in high dose group of MYQ treatment for one-month compared with I/R group (Fig. 2b). Levels of caspase-3 were not significantly different among all groups (Fig. 2c). These results indicate that MYQ may exerts its protective effects via parts of apoptotic signal pathway.

Effects of MYQ on autophagic signal pathway in rat I/R model
It is well-known that I/R could influence the Ca$^{2+}$ regulatory protein expressions [34]. Our results show that one-week after I/R, both expressions of NCX1 and Serca2a were significantly reduced compared with sham group. Treatment with MYQ for one-week could reverse these reductions (both of low and high dose). In addition, after one-month I/R, expression of NCX1 were still low, but not significantly different with sham group, and MYQ treatment also markedly increased NCX1 expressions (both of low and high dose). Furthermore, 1 month after I/R, expression of Serca2a was still markedly lower compared with sham group, and MYQ treatment also significantly increased the Serca2a expression compared with I/R group (Fig. 3). There results suggest that MYQ could up-regulate Ca$^{2+}$ handling protein expressions therefore ameliorate intracellular Ca$^{2+}$ overload in response to cardiac I/R.

Discussion
In the present study, our data clearly demonstrate that MYQ could improve cardiac function and reduce...
myocardial injury in response to long term I/R injury. The involved mechanism may be through regulation of cell apoptosis cascades (increase of Bcl2 and decrease Bax expressions), increasing Ca^{2+} handling proteins (increase of NCX1 and Serca2a expression), and regulating autophagic proteins signal pathways (decrease of Beclin-1 expression).

MYQ used in the present study is composed of five herbs, each having been widely used in Traditional Chinese Medicine for various diseases. Milkvetch Root has been ascribed to be an important herb to improve Qi [7]. BuYang HuanWu decoction, composed of Milkvetch Root and other herbs, has been reported to have protective effects on myocardial ischemia induced by isoproterenol in rats [37]. Milkvetch Root also has been reported to have specific effects on Ca^{2+} handling both in cellular level [10, 11] and whole body level [12]. Recently, protective effects of Milkvetch Root against endothelial dysfunction in hypertrophic rats induced by isoproterenol also has been reported [38]. Besides the effects of Milkvetch Root on cardiovascular diseases, its effects on other diseases also have been reported, such as malignant plural effusion [14], diabetes [39, 40], and cancer [41]. Although Semen Pharbitidis is reported to has toxicology effect after long term administration [42], its therapeutic effect on relieving constipation by purgation, dispersing phlegm and washing excessive

Table 2: Effect of MYQ on cardiac function

| HR (bpm) | Sham (n = 10) | I/R (1 week) | I/R (1 month) | Low dose (n = 10) | High dose (n = 9) | Low dose (n = 10) | High dose (n = 9) |
|----------|---------------|--------------|--------------|------------------|------------------|------------------|------------------|
| 403.8 ± 20.8 | 354.8 ± 50.8 | 374.6 ± 26.5 | 443.6 ± 18.9 | 310.8 ± 22.4 | 355.7 ± 34.0 | 3940 ± 41.8 |
| 147.9 ± 6.8 | 185.8 ± 28.3 | 163.3 ± 11.7 | 136.3 ± 6.3 | 203.7 ± 22.4 | 179.7 ± 17.0 | 1668 ± 29.1 |
| 91.7 ± 3.4 | 80.8 ± 2.2* | 70.2 ± 13.3 | 68.1 ± 4.7 | 57.5 ± 2.1* | 72.4 ± 9.2 | 82.3 ± 33 |
| 82.2 ± 2.8 | 50.0 ± 2.4* | 45.9 ± 11.2 | 54.3 ± 5.5 | 448.1 ± 10.8 | 505.0 ± 80 | 617 ± 38 |
| 86.8 ± 2.3 | 61.9 ± 18.1* | 55.9 ± 11.7 | 60.7 ± 4.9 | 50.8 ± 0.9* | 59.0 ± 82 | 70.7 ± 36 |
| 95. ± 3.7 | 30.7 ± 2.4* | 24.3 ± 42 | 13.7 ± 3.2 | 12.7 ± 25 | 21.9 ± 22 | 205 ± 11 |
| 1005.7 ± 7.5 | 876.8 ± 18.1* | 827.10 ± 5.9 | 983.6 ± 6.0* | 805.7 ± 2.1* | 908.6 ± 68 | 1055 ± 31 |
| 2.8 ± 0.9 | 5.8 ± 0.4* | 5.5 ± 19 | 5.1 ± 3.8 | 7.8 ± 1.9* | 11.0 ± 3.4 | 2.4 ± 1.9* |
| 549.2 ± 2.8 | 428.1 ± 14.1* | 405.7 ± 7.2 | 498.1 ± 1.5 | 379.1 ± 0.9* | 419.5 ± 50 | 514 ± 1.7 |
| 15.4 ± 2.3 | 41.9 ± 14.3* | 30.6 ± 13.2 | 41.0 ± 6.7 | 195.3 ± 3.7* | 186.6 ± 64 | 376 ± 3.4 |
| 67.8 ± 8.6 | 670.5 ± 52 | 641.1 ± 11.6 | 839.5 ± 5.1 | 57.3 ± 12 | 648.7 ± 73 | 870 ± 35 |
| 69.5 ± 3.8 | 461.1 ± 1.1 | 467.7 ± 7.5 | 490.9 ± 2.6 | 470.7 ± 2.7 | 502.5 ± 58 | 503 ± 1.8 |
| 33.425 ± 2.75 | 30.482 ± 40.66 | 31.914 ± 59.69 | 43.380 ± 36.97 | 25.151 ± 19.53 | 33.544 ± 50.12 | 42.123 ± 53.24 |

Table 2: cardiac function parameters. HR (Heart rate), RRI (the R-R interval), SAP (Systolic arterial pressure), DAP (Diastolic arterial pressure), MAP (Mean arterial pressure), PP (Pulse pressure), Pmax (the maximum of left ventricular pressure development), Pmin (the minimum of left ventricular pressure development), Pmean (the mean of ventricular pressure development), LVEDP (left ventricular end-diastolic pressure), P@-dp/dtmax (the left ventricular pressure corresponding to the rates of maximum negative left ventricular pressure development), CFL (cardiac force loop), At(CFL)(CFU) total area of CFL, A1(CFL)(CFU) area of the first CFL, A2(CFL)(CFU) area of the second CFL, A3(CFL)(CFU) area of the third CFL, A4(CFL)(CFU) area of the fourth CFL, As(CFL) (systolic area of CFL), Ad(CFL) (diastolic area of CFL), CRHL (contraction relaxation harmoniousness loop), Smax (CRHL) the maximum of positive left ventricular systolic pressure of d^2p/dt^2, Smin (CRHL) the maximum of negative left ventricular systolic pressure of d^2p/dt^2, Dmax (CRHL) the maximum of positive left ventricular diastolic pressure of d^2p/dt^2, Dmin (CRHL) the maximum of negative left ventricular diastolic pressure of d^2p/dt^2 were measured by a cardiac function analysis system. Values were expressed as mean ± S.E.M. Sham: Sham group; I/R: ischemic/reperfusion group. Low dose: I/R + (1 ml) MYQ treatment group. High dose: I/R + (4 ml) MYQ treatment group. *P < 0.05 compared with sham group. **P < 0.05 compared with I/R group.
fluid is also well investigated, and is widely used for the treatment of edema, ascites, hydroncus, simple obesity, lung fever, tumor and ardent fever [43–45]. Extraction of *Semen Pharbitidis* also shows antioxidant effect [46]. *Cinnamom Twig* has excellent antihyperglycemic, antioxidant, protein tyrosine phosphatase 1B inhibitory activities [17], and anti-inflammatory activities [47]. A population-based study also showed that *Cinnamom Twig* has sedative and anti-inflammatory effect for the treatment of endometriosis-related symptomatic discomfort [15]. *Fructus Amomi* is used to treat stomach disorders, pulmonary diseases and liver complaints [48, 49], which also has antioxidant activity [50]. *White Peony Root* has been reported to promote the recovery of bone marrow hemopoietic function in a myelosuppressed mouse model [51], and its therapeutic effect on radiation-induced esophageal toxicity is also has been observed [52, 53]. Based on above-mentioned literatures, composition of MYQ includes the herbs for improving cardiovascular and digestive function, and regulating immune system, which is accordance with the core concept of Wu Meng Therapy: treatment of cardiovascular diseases combined with circulatory and digestive systems. In the present study we firstly explore the therapeutic effects of MYQ in I/R induced cardiac injury, secondly, we investigate the possible mechanisms involved in.

It is well-known that reperfusion of heart results in further damage of heart tissue, and induces reduction of heart function [54, 55]. Our investigation clearly shows that there is significant reduction of cardiac function...
after one-week and one-month reperfusion, which is concordance with previous report [55]. However, MQY treatment could improve cardiac function after I/R one-week (high dose) and one-month (both low dose and high dose, Table 2). These results strongly reveal the therapeutic effect of MQY in response to I/R induced reduction of cardiac function. In addition, our results also show that tissue injury is ameliorated after treatment of MQY for one-month, although one-week treatment does not show any significant improvement. It should be noted that dosages, especially the high dose, used in the present study are well-designed to investigated the mechanisms involved in. Also, it should be mentioned that time of MQY treatment is more similar for treatment of clinical patients with cardiac infarction patients, which might provide more precise evidence for clinical application.

Fig. 3 Effects of MYQ on the expression of NCX1 and SERCA2a. a Expression of SERCA2a, upper is the representative blots of NCX1 and GAPDH; lower is the densitometric analysis of NCX1 expression normalized to GAPDH (n = 8). b Expression of SERCA2a, upper is the representative blots of SERCA2a and GAPDH; lower is the densitometric analysis of SERCA2a expression normalized to GAPDH (n = 8). Sham: Sham group; I/R: ischemic/reperfusion group. I/R + Low dose: Low dose (1 ml, P.O. tid for one-week or 1 month) MYQ treatment group. I/R + High dose: High dose (4 ml, P.O. tid for one-week or 1 month) MYQ treatment group. All data were expression as mean ± S.E.M. † P < 0.05 compared with sham group, * P < 0.05 compared with I/R group.

Fig. 4 Effects of MYQ on the expression of Beclin-1 and LC3. a Expression of Beclin-1, upper is the representative blots of Beclin-1 and GAPDH; lower is the densitometric analysis of Beclin-1 expression normalized to GAPDH (n = 8). b Expression of LC3, upper is the representative blots of LC3 I and LC3 II; lower is the densitometric analysis of the ratio of LC3 II to LC3 I (n = 8). Sham: Sham group; I/R: ischemic/reperfusion group. I/R + Low dose: Low dose (1 ml, P.O. tid for one-week or 1 month) MYQ treatment group. I/R + High dose: High dose (4 ml, P.O. tid for one-week or 1 month) MYQ treatment group. All data were expression as mean ± S.E.M. † P < 0.05 compared with sham group.
Traditionally, it has been well recognized that cellular responses to I/R is highly related to the activation of apoptotic pathways and various strategies were explored to suppress the activation of apoptosis [56, 57]. Although our present data does not show any markedly effects of MQY on apoptosis signal pathway with low dose, however, high dose of MQY shows significant effect on regulation of anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax, which implies that MQY exerts its protective effect may be through regulation of apoptotic signal pathway. Interestingly, expression of caspase-3, which is best known for its role in the execution phase of apoptosis, is not different after one-week or one-month reperfusion compared with control group. We speculate that caspase signal pathways only play pivotal role in apoptosis-induced cardiac injury, which may be through regulation of apoptotic proteins, cytosolic Ca$^{2+}$ handling proteins and autophagic proteins signal pathways.

In conclusion, our present observation demonstrates that MYQ have potential therapeutic effects on I/R-induced cardiac injury, which may be through regulation of apoptotic proteins, cytosolic Ca$^{2+}$ handling proteins and autophagic proteins signal pathways.

**Abbreviations**
IR: Ischemia-reperfusion; MYQ: Modified Yi Qi decoction; NCX1: Sodium calcium exchanger; Serca2a: Sarcoplasmic reticulum Ca2+ -ATPase

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**Availability of data and materials**
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Authors’ contributions**
XY performed the experiments, X-DZ conceived the project and performed the experiments, R-QB and J-YY analyzed the data, G-XZ and J-WC designed the experiments, R-QB and J-YY analyzed the data, G-XZ and J-WC designed the experiments and wrote the final manuscript.

**Competing interests**
The authors declare that they have no competing interests.

**Consent for publication**
Not applicable.

**Ethics approval**
The present study was approved by and performed according to guidelines for the care and use of animals established by Soochow University of Animal Care and Use Committee.

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