QiShenYiQi Pills® prevents cardiac ischemia–reperfusion injury via energy modulation

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ABSTRACT

Background: QiShenYiQi Pills® (QSYQ) is a compound Chinese medicine used in China for alleviating cardiac function. The present study was designed to explore the effect and mechanism of QSYQ on ischemia–reperfusion (I/R)-induced disorders in myocardial structure and function, with particularly focusing on the regulation of energy metabolism.

Methods: Sprague–Dawley rats, with or without QSYQ pretreatment, were subjected to 30 min occlusion of the left anterior descending coronary artery and followed by 90 min or 24 h reperfusion. Myocardial blood flow (MBF) and cardiac function were evaluated at baseline, immediately after ischemia and 30, 60, 90 min, and 24 h after reperfusion. Myocardial infarction, myocardial histology and ultrastructure were assessed. Double staining of alpha-cardiac actinin and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling was conducted to assess myocardial apoptosis. ATP, ADP and AMP content was determined by Enzyme-Linked Immunosorbent Assay, F-actin in myocardial cells determined by immunofluorescence microscopy and expression of ATP synthase α, ATP5D, and phosphorylated-Myosin Light Chain (P-MLC) determined by western blotting.

Results: Pre-treatment with QSYQ protected against I/R-induced MBF decrease, myocardial infarction and apoptosis at 90 min and 24 h after reperfusion. Moreover, I/R 90 min caused an impairment on cardiac function, a decrease in the ratio of ADP/ATP and AMP/ATP, accompanying with reduction of ATP 5D expression and increase in the expression of P-MLC, meanwhile, myocardium to exhibit myocardial fiber rupture, interstitial edema, and mitochondria swelling, all of which were significantly ameliorated by pre-treatment with QSYQ.

Conclusions: The results of the present study suggest an involvement of regulation of energy metabolism in the action of QSYQ to protect against myocardial I/R injury.

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1. Introduction

Interventional therapies have been the main treatment for acute coronary syndrome, but they have not decreased the risk of serious heart events because of ischemia–reperfusion (I/R) injury [1]. I/R injury is evoked by ischemic hypoxia and reperfusion-induced peroxide. The ATP depletion by ischemic hypoxia initiates the reactive oxygen species (ROS) production after reperfusion, which exaggerates myocardium and endothelial cell injury [2]. Researchers currently focus mainly on interference in the production of peroxide [3], the expression of adhesion molecules [4], the release of inflammatory factors [5,6], and the apoptosis of cardiac myocytes [7], but the attempts so far made are far from success in clinic.

ATP is vital for myocardial cell contractility and survival. Lack of ATP is well accepted as an essential episode in I/R injury, which emerges during ischemia, and remains over the reperfusion [8]. Shortage of ATP is known to play a dual role in the pathogenesis of I/R injury, in addition to trigger ROS production, it leads to degradation of F-actin, and phosphorylation of Myosin Light Chain (MLC), the major component of thin filament and thick filament, respectively [9], and thus the abnormality of cardiac structure and function. It is well recognized

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that hypoxia during myocardial ischemia uncouples oxidative phos-
phorylation from the respiratory chain, which contributes to the cessa-
tion of ATP synthesis. On the other hand, it is unclear at present whether mitochondrial ATP synthase, the major player in ATP synthesis, is also modulated by I/R.

QiShenYiQi Pills® (QSYQ) is a compound Chinese medicine ap-
proved by China State Food and Drug Administration in 2003 for
the treatment of cardiac dysfunction [10]. The pharmacokinetics’ results showed that QSYQ reaches its maximal concentrations in blood with-
in 1 h after oral administration. Our previous study demonstrated that treatment with QSYQ attenuates pressure over-load-induced cardiac hypertrophy and myocardial fibrosis through interfering in inflammatory process [11]. QSYQ was also reported to attenuate rat I/R-induced myocardial fibrosis [12]. Furthermore, study by proteo-
tic technique has shown that myocardial protective effects of QSYQ in myocardial infarction process might be closely related to the recov-
ery of energy supply, in addition to the reduction of oxidative stress
[13]. The present study was to explore the effect of QSYQ on I/R-induced
impairment on myocardial structure and cardiac function, and the
possible implication of the energy metabolism modulation in the
QSYQ effect.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 240 to 260 g, were purchased from the Ani-
mal Center of Peking University (Certificate no. SCXK (Jing) 2006-0008). The rats were housed in cages at temperature 22 ± 2 °C, humidity 40 ± 5%, under a 12-hour light-
dark cycle, and received standard diet and water ad libitum. The rats were fasted for 12 h before experiment but allowed to access water freely. The investigations conformed to the EU adopted Directive 2010/63/EU and Guide of Peking University An-
imal Research Committee. Experiment protocols were approved by Peking University Biomedical Ethics Committee Experimental Animal Ethics Branch (LA2010-001). The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [14].

2.2. Drug and reagents

QiShenYiQi Pills® (QSYQ, Batch number: 20090105) was obtained from Tasyi Phar-
maceutical Co. Ltd, (Tianjin, China), which was produced according to the guide-
lines of Good Manufacturing Practice and Good Laboratory Practice, and the content of its major components was determined by HPLC finger print [11]. It was dissolved in saline to make a solution at concentration of 30 mg/ml, 150 mg/ml, and 300 mg/ml before experiment.

Evans blue was purchased from Sigma Ltd, freshly prepared to 0.35% solution with saline before experiment. 2, 3, 5-triphenyltetrazolium chloride (TTC) was from AMRESCO Ltd. (Cuyahoga, Ohio, USA) and prepared to 0.375% solution with phosphate buffer. Pentobarbital sodium was purchased from Beijing Chemical Agent Ltd. ATP, ADP and AMP ELISA Kits were from Beijing Huanya Biomedicine Technology Co. Ltd. The anti-
tibody against P-MLC was bought from CST (Boston, Massachusetts, USA). The antibody against ATP synthase α was from BD (Hercules, California, USA), the antibody against ATP5D from Santa Cruz Biotechnology Co., Ltd (Santa Cruz, California, USA), and the antibody against mitochon-drial ATP synthase, the major player in ATP synthesis, is also modulated by I/R.

2.3. Myocardial I/R model and animal grouping

Rats were divided into Sham group, QSYQ 0.6 group, I/R group, QSYQ 0.12 + I/R group, and QSYQ 0.6 + I/R group. 3 or 6 animals in each group for determination of each parameter. (See Tables 1 and 2 for detail). Animals were anesthetized with 2% pentobarbital sodium (60 mg/kg) by peritoneal injection, and placed in a supine posi-
tion. A tracheal cannula was inserted via mouth, with one end being connected with an animal breathing apparatus (ALC-89, Shanghai Alicot Biotech Co., China), which was set at the breathing ratio 1:1, the frequency 75/min, and tidal volume 12 ml/kg. A thor-
acotomy was performed to expose the heart, and the proximal left anterior descending coronary artery (LADCA) was ligated with a 5/0 silk. The suture silk was re-
leased after 30 min, allowing reperfusion to occur. The animals in Sham and QSYQ 0.6 group underwent the same procedure but without ligation of suture silk [15].

Ninety minutes before ischemia, the animals in QSYQ pretreatment groups were administered through gavage with QSYQ in saline at dose of 0.12 g/kg (QSYQ 0.12 + I/R group), 0.6 g/kg (QSYQ 0.6 group and QSYQ 0.6 + I/R group), or 1.2 g/kg (QSYQ 1.2 + I/R group). The animals in Sham group and I/R group received saline at 4 ml/kg.

2.4. Myocardial blood flow

After left thoracotomy, myocardial blood flow (MBF) was measured by using Laser-Doppler Perfusion Imager (PeriScan PIM3 System; PERIMED, Stockholm, Sweden) equipped with a computer at baseline, immediately after ischemia, and 30, 60, 90 min, and 24 h after reperfusion. Heart was exposed and a computer-controlled optical scanner directed a low-powered He–Ne laser beam over the exposed heart. The scanner head was positioned in parallel to the surface of heart at a distance of 18 cm. At each measuring site, the beam illuminated the tissue to a depth of 0.5 mm. A color-coded image to denote specific relative perfusion level was displayed on a video monitor, and all images were evaluated with the software LDPWin 3.1 (PeriScan PIM3 System; PERIMED, Stockholm, Sweden). The magnitude of MBF is represented by different colors, with blue to red denoting low to high. Results for the time points be-
fore 90 min after reperfusion were expressed as percentages of the baseline MBF, and for 24 h after reperfusion were presented as Perfusion Units [15].

2.5. Heart function test

Cannulation was inserted into left ventricle through right carotid artery, which was connected to a bio-function experiment system BL-420F (Chengdu Taimen Technology Ltd., Chengdu, China). Heart rate (HR), left ventricular systolic pressure (LVP), left ventricular diastolic pressure (LVEDP), left ventricular end diastolic pressure (LVESP), left ventricular maximum upstroke velocity (+dp/dtmax), and left ventricular maximum descent velocity (–dp/dtmax) were measured at baseline, immediately after ischemia, and 30, 60, 90 min after reperfusion with BL-420F equipment [16].

2.6. Myocardial infarct size

At 90 min and 24 h after reperfusion, left anterior descending coronary artery was
ligated, and 2 ml of 0.35% Evans blue was administrated through femoral vein. Hearts were rapidly excised and sliced into 5 sections (1 mm thick), parallel to the atrioven-
tricular groove, from the apex cordis to the ligation site. Slices were incubated for 15 min at 37 °C in a 0.375% solution of TTC, and then photographed with a stereoscope scanner head was positioned in parallel to the surface of heart at a distance of 18 cm. At each measuring site, the beam illuminated the tissue to a depth of 0.5 mm. A color-coded image to denote specific relative perfusion level was displayed on a video monitor, and all images were evaluated with the software LDPWin 3.1 (PeriScan PIM3 System; PERIMED, Stockholm, Sweden). The magnitude of MBF is represented by different colors, with blue to red denoting low to high. Results for the time points be-
fore 90 min after reperfusion were expressed as percentages of the baseline MBF, and for 24 h after reperfusion were presented as Perfusion Units [15].

The same animals were used for detection of myocardial blood flow and myocardial infarct size, and the same is for detection of hemodynamics and ATP/ADP/AMP content assay. Sham: Sham group; QSYQ 0.6: QSYQ 0.6 g/kg plus Sham group; I/R: I/R 90 min group; QSYQ 0.12 + I/R: pre-treatment with QSYQ at 0.12 g/kg plus I/R 90 min group; QSYQ 0.6 + I/R: pre-treatment with QSYQ at 0.6 g/kg plus I/R 90 min group; QSYQ 1.2 + I/R: pre-treatment with QSYQ at 1.2 g/kg plus I/R 90 min group.

Table 1

<table>
<thead>
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<th>Number of animals for different experimental groups and various parameters at 90 min after reperfusion.</th>
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<tr>
<td>QSYQ 0.6</td>
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<td>Sham</td>
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<td>QSYQ 0.6</td>
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<td>QSYQ 0.6 + I/R</td>
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The same animals were used for detection of myocardial blood flow and myocardial infarct size. The present study was to explore the effect of QSYQ on I/R-induced impairment on myocardial structure and cardiac function, and the possible implication of the energy metabolism modulation in the QSYQ effect.

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was stained white, area at risk (AAR) was pink, while non-infarction zone was blue. Myocardial infarct size, AAR and left ventricle size (LV) were analyzed on each slice by Image-Pro Plus 6.0 (Media Cybernetic, Bethesda, MD, USA) \((n=6)\). The ratios of AAR/LV (%) and infarct area/AAR (%) were calculated, and the values from 5 slices were averaged and used to express the degree of myocardial infarction \[17\].

2.7. Myocardial histology

At 90 min after reperfusion, thoracotomy was performed, and heart was removed \((n=3)\), fixed in 4% paraformaldehyde (PFA) solution for 4 h for processing paraffin section. The paraffin sections \((5 \mu m)\) were stained with hematoxylin eosin (HE) as routine \[18\].

2.8. Double staining of alpha-cardiac actinin and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)

At 90 min and 24 h after reperfusion, heart was perfused with saline, and then removed and fixed in 4% PFA solution for 48 h for preparation of paraffin section. The double staining of alpha-cardiac actinin and TUNEL were incubated overnight at 4 °C with a specific antibody against sarcomeric alpha actinin (Abcam, Cambridge, USA), and then the specific antibody was detected by incubation with DyLight™-labeled secondary antibody (KPL, Gaithersburg, Maryland, USA). Then TUNEL staining was undertaken by a cell death detection kit (Roche, Basel, Switzerland), according to the manufacture's instruction, and the nuclei were labeled with Hoechest 33342. Five fields were selected from the surrounding infarction areas of the left ventricle for each section at ×40 magnification of objective, and observed with a Laser Scanning Confocal Microscope (TCS SP5, Leica, Mannheim, Germany). The numbers of the TUNEL-positive cells in the five fields were counted, and the average was calculated and expressed as cell number per field.

2.9. Immunofluorescence staining and confocal microscopy

At 90 min after reperfusion, the heart was perfused with saline and a tissue block at the middle one-third between the apex and the ligation point was cut and fixed in 4% PFA solution, and 10 μm frozen sections were prepared. After the sections were completely air dried, myocardial F-actin was labeled with rhodamine phalloidin (R415, Invitrogen, Carlshad, California, USA). A Laser Scanning Confocal Microscope (TCS SP5, Leica, Mannheim, Germany) was applied to examine the immunofluorescence staining.

2.10. Ultrastructure examination

Rats were sacrificed under anesthesia 90 min after reperfusion, and the hearts were removed \((n=3)\). Two approximately 1 mm³ fresh myocardial tissue blocks were cut at a 4 mm level above the apex cordis from the surrounding infarct region of left ventricle. The tissue blocks were fixed with 3% glutaraldehyde overnight at 4 °C, washed 3 times with phosphate-buffered solution, and then post-fixed with 1% osmium tetroxide for 2 h. The ultrathin sections were prepared as routine, stained with uranyl acetate and lead citrate, then observed and photographed with a transmission electron microscope (JEM 1230, JEOL, Tokyo, Japan).

2.11. Assessment of energy metabolism

At 90 min after reperfusion, rats were perfused with saline under anesthesia, and the hearts were removed \((n=6)\). The tissue from left ventricle was sampled at about 2 mm under ligature, quickly frozen in liquid nitrogen, and stored at −80 °C for a maximum of 1 week before use. The whole protein of the tissues was extracted with a protein extraction kit (Applygen Technologies, Beijing, China), according to the manufacture's instruction. Briefly, eighty to one hundred milligrams of tissue was cut into pieces, mixed with 1 ml of RIPA containing 5 g/ml leupeptin, 5 g/ml aprotinin, 5 μg/ml pepstatin, and 5 mM PMSF. The mixture was homogenized, incubated on ice for 30 min, and centrifuged at 19357 g, 4 °C, for 10 min. The resultant supernatant was taken as whole protein.

The content of ATP, ADP and AMP of myocardium was assessed with ELISA by microplate reader (Multiskan MK3, Thermo, USA), according to the manufacture's instruction.

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**Fig. 1.** The effect of QSYQ pre-treatment on MBF of rats. A: MBF images of rats subjected to I/R 90 min acquired by Laser Scanning Doppler Perfusion Imager in Sham, QSYQ0.6, I/R 90 min, QSYQ 0.12 + I/R 90 min, QSYQ 0.6 + I/R 90 min, and QSYQ 1.2 + I/R 90 min group at Baseline, 0 min, 30 min, 60 min and 90 min after reperfusion. B: Time course of MBF of rats subjected to I/R 90 min in various groups. The linear mixed effect models were analyzed for repeated measurement data, and least squares means were calculated between the groups of different time points. Values are means±SEM \((n=6)\). *\(P<0.05\) vs. Sham group. #\(P<0.05\) vs. I/R 90 min group. C: Representative color images of MBF of rats subjected to I/R 24 h acquired by Laser-Doppler Perfusion Imager. D: Quantitative evaluation of MBF of rats subjected to I/R 24 h. Data are mean±SEM. *\(P<0.05\) vs. Sham group, #\(P=0.05\) vs. I/R 24 h group.
2.12. Western blotting assay

Rats were sacrificed 90 min after reperfusion, and 200 mg of myocardial tissue was sampled from the surrounding of infarct area of left ventricle, and stored at −80 °C (n = 3). The whole protein was extracted as described above. The concentration of whole protein was determined with a BCA protein assay kit (Applygen Technologies, Beijing, China), according to the manufacture's instruction. For each sample, the assessment was undertaken twice, taking the average as the concentration. The protein concentration was equalized to the lowest one detected.

The whole protein was mixed with 2 fold electrophoresis sample buffer. After separated on 12% SDS-PAGE, the proteins were transferred to polyvinylidene difluoride membrane. After 1 h blocking with 5% nonfat dry milk or 5% BSA, rinsing with TBS-Tween for 3 times, 5 min each, the membrane with target proteins was cut and incubated overnight at 4 °C with following antibodies: antibody against P-MLC (1:1000, Cell Signaling Technology, MA, USA), ATP synthase α (1:1000, BD, NJ, USA), ATP5D (1:200, Santa Cruz Biotechnology, California, USA). Afterward, the membranes were rinsed 3 times, 5 min each, incubated with secondary antibody for 1 h at room temperature, followed by rinsing with TBS-Tween 3 times, 10 min each time. The protein was quantified by scanning densitometry in the X-film using a bio-image analysis system (Image-Pro plus 6.0, Media Cybernetics, Bethesda, Maryland, USA). The result of each group was expressed as a relative optical density compared with that from Sham group.

2.13. Statistical analysis

All data were expressed as mean±SE. Statistical analysis was carried out with SAS 9.3 statistical software, and one-way analysis of variance was used, and then for post hoc testing, Fisher’s least-significant-difference test was used for multiple comparisons between groups. For repeated measurement data, the linear mixed effect models were analyzed, and least squares means were calculated between the groups of different time points. P < 0.05 was considered as statistically significant.

3. Results

3.1. Changes in MBF

Fig. 1A shows the color images acquired by the Laser Scanning Doppler before 90 min after reperfusion in the five groups at different time point. No obvious difference in MBF at baseline was observed among the five groups, no apparent difference in MBF between QSYQ 0.6 group and Sham group at each time point, either. A prominent decrease in MBF occurred in I/R 90 min group immediately after ischemia, and persisted till the end of the observation. QSYQ pre-treatment prevented MBF from decrease by I/R 90 min, with the higher dose being more efficient than lower dose.

Fig. 1B is the time courses of MBF changes in the five groups, which confirmed the impression from Fig. 1A. Particularly, the MBF in I/R 90 min group decreased 40% of baseline after ischemia, which had no recovery over reperfusion. MBFs in QSYQ pre-treatment groups followed a time course similar to that in the I/R 90 min group, but with a significant higher MBF being observed for all the doses tested at 30 min, and for QSYQ 0.6 g/kg and QSYQ 1.2 g/kg at 60 and 90 min after reperfusion, as compared to I/R 90 min group.

Fig. 1C shows the effect of QSYQ on MBF at 24 h after reperfusion. The MBF decreased in I/R 24 h group, compared with Sham group. Pre-treatment with QSYQ obviously attenuated I/R-induced decrease in MBF. The above results were confirmed by quantitative evaluation, as shown in Fig. 1D.

Fig. 2. The effect of QSYQ pre-treatment on myocardial infarct size of rats. A: Representative five slices of left ventricle (LV) stained by Evans blue-TTC in Sham (a), QSYQ 0.6 (b), I/R (c), QSYQ 0.12 + I/R (d), QSYQ 0.6 + I/R (e), and QSYQ 1.2 + I/R (f) group at 90 min after reperfusion. B and C: Quantitative measurement of AAR/LV and infarct area/AAR area after I/R 90 min in various groups, respectively. Values are means±SEM (n = 6). *P < 0.05 vs. Sham group, #P < 0.05 vs. I/R 90 min group. D: Representative slices of ventricle stained by Evans blue-TTC at 24 h after reperfusion. a: Sham group; b: QSYQ 0.6 group; c: I/R 24 h group; d: QSYQ 0.6 + I/R 24 h group. E and F: Quantitative analysis of AAR/LV and infarct area/AAR area after I/R 24 h. Data are mean±SEM. *P < 0.05 vs. Sham group, #P < 0.05 vs. I/R 24 h group.

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Fig. 3. The effect of QSYQ pre-treatment on the energy metabolism in the myocardium of rats subjected to I/R 90 min. A and B: The effects of QSYQ on the ratio of ADP/ATP, and AMP/ATP in myocardium in Sham group, QSYQ 0.6 group, I/R 90 min group and QSYQ 0.6 + I/R 90 min group, respectively. Values are means ± SEM (n = 6). *P < 0.05 vs. Sham group, #P < 0.05 vs. I/R 90 min group. C: Representative Western blotting bands and semi-quantitative analysis of ATP synthase α (C1) and ATP 5D (C2) in various groups. Values are means ± SEM (n = 3). *P < 0.05 vs. Sham group, #P < 0.05 vs. I/R 90 min group. D: Representative Western blotting band and semi-quantitative analysis of P-MLC in various groups. Values are means ± SEM (n = 3). *P < 0.05 vs. Sham group, #P < 0.05 vs. I/R 90 min group.

Fig. 4. The effect of QSYQ pre-treatment on myocardial histology and myocardial ultrastructure in rats after I/R 90 min. A: Representative photographs of myocardium by HE. Bar = 50 μm. a: Disrupted myocardial fiber, b: edema, and c: infiltrated leukocytes. B: Representative photographs of F-actin stained by rhodamine phalloidin. a: Disrupted myocardial fiber. Bar = 10 μm. C: Presented are the representative electron micrographs from Sham group, QSYQ 0.6 group, I/R group, and QSYQ 0.6 + I/R group. a: Myofilibril, b: mitochondria, c: disrupted myofilibril, and d: swelled mitochondria. 1. Sham group; 2. QSYQ 0.6 group; 3. I/R 90 min group; and 4. QSYQ 0.6 + I/R 90 min group (n = 3).
3.2. Changes in myocardial infarct size

Myocardial infarct was assessed 90 min and 24 h after reperfusion by Evans blue-TTC staining, and the representative heart slices in different groups are shown in Fig. 2A and D, wherein the pink area represents ischemic myocardial tissue, while the white area represents the infarction region. Apparently, myocardial tissue slices from Sham group and QSYQ control group exhibited no ischemia and infarct. In contrast, noticeable ischemia and infarct areas were observed in myocardial tissue slices both in I/R 90 min group and in I/R 24 h group. As compared to I/R group, on the other hand, heart slices from QSYQ pre-treatment groups had a obviously smaller area of myocardial infarct, but a similar area of ischemic region.

This impression is corroborated by the quantitative analysis of AAR/LV and infarct area/AAR, as shown in Fig. 2B and C, and E and F, respectively. Ninety minutes after reperfusion, AAR/LV increased obviously in I/R group compared with Sham, but with no significant difference being found between I/R and QSYQ pre-treatment groups. Infarct area/AAR in I/R group increased pronouncedly as well in comparison with Sham group and QSYQ alone group, which, however, was prevented significantly by pretreatment with QSYQ in a dose-dependent manner. The effect of QSYQ on myocardial infarction at 24 h after reperfusion exhibited a comparable pattern to that of 90 min after reperfusion.

3.3. Energy metabolism

To address the energy metabolism in different conditions, we first explored the ratio ADP/ATP and AMP/ATP in cardiac tissue. As shown in Fig. 3A and B, when compared with Sham group, QSYQ pre-treatment alone had no effect on either ADP/ATP or AMP/ATP. Of notice, I/R 90 min challenge dramatically increased ADP/ATP and AMP/ATP, to one and two fold Sham group, respectively, indicating a perturbation in the balance of energy metabolism inclining toward ATP catabolism. Interestingly, pre-treatment with QSYQ at 0.6 g/kg significantly prevented both ADP/ATP and AMP/ATP from elevation by I/R 90 min.

We next determined the expression of ATP 5D and ATP synthase α, two of the subunits of ATP synthase, in cardiac tissue in response to I/R 90 min, and the role of QSYQ in regulation of their expression. As shown in Fig. 3C1, the expression of ATP 5D reduced significantly after I/R, as compared to Sham group. Pre-treatment with QSYQ at the dose of 0.6 g/kg significantly restrained the decline of ATP 5D expression evoked by I/R. There was no notable variation in the expression of ATP synthase α among the four groups (Fig. 3C1).

As one of the important effectors of ATP, the phosphorylation of MLC in various conditions was assessed as well. As expected, the P-MLC expression varied among the four groups (Fig. 3D) as the ratio of ADP/ATP and AMP/ATP did (Fig. 3A and B).

3.4. Myocardium histology and myocardial ultrastructure

To gain insight into the effect of QSYQ pre-treatment on the I/R-induced alteration in myocardium structure, we first examined the histology of myocardium in different groups (Fig. 4A). Compared with Sham group, distinct alterations occurred in the surrounding infarction areas of myocardial tissues from I/R group, including myocardial interstitial edema, rupture of myocardial fibers, and infiltration of leukocytes. However, pretreatment with QSYQ at the dose of 0.6 g/kg ameliorated the I/R-induced myocardial alterations, particularly interstitial edema and myocardial fiber disruption.

Fig. 5. The effect of QSYQ pre-treatment on myocardial apoptosis. A, C: Presented are the representative photographs of double staining of alpha-cardiac actinin and TUNEL. Nucleus are stained with blue, alpha-cardiac actinin red, and TUNEL-positive cells green (arrows), Bar=25 μm. n = 6. B, D: Quantitative analysis of apoptosis cells among the various groups. Ordinates are cell number per field. Values are means±SEM (n = 6). *P<0.05 vs. Sham group, #P<0.05 vs. I/R group.
The results of rhodamine phallolidin-labeled F-actin in Fig. 4B further confirmed the above results. Apparently, the I/R-induced F-actin decrease and myocardium rupture were protected from in QSYQ 0.6 + I/R group.

The representative ultrastructural images of myocardium in the four groups are presented in Fig. 4C. Of notice, the myocardium in Sham group displayed a normal ultrastructure with regularly myofibrils, well preserved sarcomeres and densely packed mitochondria. The I/R challenge provoked a dramatic injury in myocardium ultrastructure, manifested as disrupted myofibrils and swelling mitochondria. These changes were alleviated by pre-treatment with QSYQ at 0.6 g/kg.

3.5. Myocardial apoptosis

Fig. 5 shows the images of double staining of alpha-cardiac actinin and TUNEL in surrounding infarction areas of the left ventricle myocardium from the various groups, wherein nuclei were stained blue, alpha-cardiac actinin red, and TUNEL-positive cells green. In Sham group, few TUNEL-positive cells were observed in the myocardium. On the contrary, at both 90 min and 24 h after reperfusion, a large number of TUNEL-positive cells were observed in I/R groups, while apparent less TUNEL-positive cells were visible in QSYQ 0.6 + I/R group.

3.6. Heart function

Heart function was assessed in the four groups to evaluate the role of QSYQ in preventing heart from I/R injury. As noticed in Fig. 6 in comparison with Sham group, I/R caused a significant decline in LVSP and +dp/dtmax, and an increment in LVDP, LVEDP and −dp/dtmax, indicating an impairment on heart function. Evidently, these impairments were protected from by pre-treatment with QSYQ at 0.6 g/kg, except for that in LVDP.

4. Discussion

The present study revealed that pre-treatment with QSYQ protected heart from I/R-induced impairment, including the decrease in coronary blood flow, myocardial infarction, the degradation of myocardial F-actin and phosphorylation of myocardial MLC, the rupture of myocardial fibers, and the diminution in left ventricular contractility. Importantly, the evidence from the present study shows that disorder in one of the ATP synthase subunits, ATP 5D, may participate in depleting ATP during I/R, and this disorder is presumably prevented from by QSYQ pretreatment.

QSYQ is currently used in China to cope with heart troubles. However, the study in animal model regarding its role in management of cardiac dysfunction is limited. We have previously reported that post-treatment with QSYQ attenuates pressure over-load-induced rat cardiac hypertrophy and myocardial fibrosis, and proposed that it exerts this action through interfering in inflammatory process [11]. In the myocardial I/R injury model in present study, the anti-inflammatory potential of QSYQ is further documented, as shown by the attenuation of I/R-induced leukocyte infiltration after QSYQ pre-treatment. Moreover, some additional benefits of QYSQ were observed in the present study, including diminishing the number of TUNEL-positive cells and the expression of P-MLC, and increasing F-actin in cardiac myocytes. Obviously, protection of cardiac myocytes from apoptosis contributes to the attenuation effect of QSYQ on myocardial infarction; while QSYQ may
The results provide a novel treatment strategy against I/R-induced myocardial injury. More importantly, QSYQ was revealed to protect against the decrease in ATP 5D level after I/R. Furthermore, QSYQ alone did not alter the expression of ATP synthase, did not change at all. Rather, decrease in ATP 5D level may be caused by a disorder somewhere in the process of its expression.

The present study still has some limitations. Firstly, the infarct size was evaluated up to 24 h after reperfusion, while cardiac function was assessed only up to 90 min after reperfusion. Long term protective effect of QSYQ on cardiac structure and function needs to be further explored. Secondly, the experiments were conducted on rats in current study, and large animal models are needed in further study so that the results obtained may be even closer to the clinic outcomes. Finally, in view of the complexity of QSYQ ingredients, more studies are required to better understanding the mechanism whereby the compound works.

In conclusion, pre-treatment with QSYQ could protect myocardial structure and cardiac function from I/R injury, which may be associated with its potential to protect the expression of ATP 5D from injury by I/R. The results provide a novel treatment strategy against I/R-induced myocardial injury and microcirculatory disturbance, and further studies are needed to address the benefit of QSYQ as an adjunctive therapy for coronary reperfusion.

References


