

Full-length article

Metallothionein mediates cardioprotection of isoliquiritigenin against ischemia-reperfusion through JAK2/STAT3 activationWei AN, Jing YANG¹, Ying AO*Department of Pharmacology, School of Medicine, Wuhan University, Wuhan 430071, China***Key words**

isoliquiritigenin; myocardial ischemia-reperfusion; metallothionein; janus kinase; STAT3 transcription factors

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Abstract

Aim: To examine whether isoliquiritigenin (ISL) can attenuate myocardial ischemia-reperfusion (MI/R) injury in rats by inducing metallothionein (MT) through activation of janus kinase 2 (JAK 2)/signal transducers and activators of transcription 3 (STAT 3) pathway. **Methods:** The experimental model of MI/R in rats was generated by 30 min of ischemia and reperfusion for 2 h. The mRNA expression of MT, COX-2, and iNOS were measured by RT-PCR. The protein expressions of MT, JAK/STAT, extracellular signal-regulated kinase (ERK), and Akt were determined by Western blotting in the absence or presence of a JAK kinase inhibitor, tyrphostin AG490 (1.0 mg/kg, iv, 1 h before ischemia). **Results:** Pretreatment with ISL markedly decreased the severity of reperfusion-induced arrhythmias and myocardial infarct size. In the ISL 20 mg/kg group, the activities of lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) were reduced by 38.4% and 51.3% when compared with the vehicle group. Increased JAK 2/STAT 3 phosphorylation was accompanied by increased synthesis of MT but not of COX-2 or iNOS in ISL-treated groups. AG490 can significantly weaken ISL-induced cardioprotection and prevent the increase of MT expression and JAK 2/STAT 3 phosphorylation. **Conclusion:** ISL protected MI/R injury through activation of JAK 2/STAT 3 signal transduction pathway, which might be involved in mediating the upregulation of MT expression.

Introduction

Myocardial infarction is one of the major causes of death in many countries. Although restoration of blood flow is the only way to save the myocardium from necrosis, reperfusion-induced injuries with the help of thrombolytic therapy are at the background of a high mortality rate^[1]. Extensive studies show that myocardial ischemia-reperfusion (MI/R) injury is associated with increased generation of reactive oxygen species (ROS). These oxygen free radicals may result in depressions in contractile function, arrhythmias, depletion of endogenous antioxidant network, and membrane permeability changes^[2].

In recent years, accumulating experiments have shown that metallothionein (MT), a low molecular non-enzyme protein, is a strong endogenous cytoprotective agent against cardiovascular injury. It plays an important role in detoxify-

ing against harmful heavy metals, scavenging oxygen radicals, and stabilizing biomembranes^[3]. The antioxidant function of MT was first suggested in the early 1980s. Studies *in vitro* have revealed that MT reacts directly with reactive oxygen species, including superoxide, hydroxyl, and hydrogen peroxide. In particular, the antioxidant function of MT in the heart has been explored extensively. The data gathered from recent studies using a cardiac-specific, MT-overexpressing transgenic mouse have provided direct evidence to support this physiological role of MT^[4]. Under acute and chronic oxidative stress conditions such as treatment with doxorubicin, ischemia-reperfusion, and dietary copper-induced restriction, MT-overexpressing transgenic mouse hearts displayed a marked resistance to the injurious consequences, including biochemical, pathological, and functional alterations. This protective action of MT correlates with its inhibition of reactive oxygen species-induced

lipid peroxidation^[5]. Exogenous administration with MT or induction of endogenous MT production may be used as a new strategy in prevention and therapy of cardiovascular diseases^[6,7].

The janus kinase/signal transducer and activator of transduction (JAK/STAT) pathway is a newly discovered intracellular signal-transducing pathway that is activated by oxygen radicals, various cytokines, and growth factors in ischemic heart diseases. Recent studies have demonstrated that MI/R induce rapid activation of this pathway^[4]. Although the functional consequences of this event remains to be elucidated, there is emerging evidence that JAK/STAT signaling plays an important role in the development of the cardioprotected phenotype associated with ischemic preconditioning.

Some studies *in vivo* also indicated that activation of STAT 3 protects myocardium from ischemia-reperfusion injury at least partially through metallothionein, including MT-1 and MT-2^[8]. Specifically, brief episodes of MI/R activated JAK 1 and JAK 2, followed by recruitment of STAT 1 and STAT 3, resulting in transcriptional upregulation of MT^[9,10].

Flavonoids are ubiquitous compounds found in a wide variety of edible plants, fruits, vegetables, grains and red wine. They are an integral part of the human diet, and are important and effective constituents of some medicines, especially of Chinese herbal medicines^[11]. Epidemiological studies have indicated an association between the consumption of flavonoids and a reduced incidence of diseases such as stroke^[12], coronary heart diseases^[13] and cancer^[14]. *Glycyrrhiza glabra* (family: Leguminosae), the licorice plant, is widely used in Chinese medicine and food and has a history of consumption for the past 6000 years. Isoliquiritigenin (ISL), 2',4',4'-three hydroxy chalcone, one of the components in the root of *Glycyrrhiza glabra*, is a member of the flavonoids, which have shown various biochemical activities, such as vasorelaxant effect, antioxidant, anti-platelet, anti-tumor, anti-allergic, antiviral activities and estrogenic properties^[15]. However, the effects of ISL on MI/R injury have not been studied. Therefore, the protective effects of ISL and its molecular mechanism were investigated by using coronary artery occlusion–reperfusion rat model *in vivo*, which is considered to more closely mimic the clinical situation.

Materials and methods

Reagents The roots of *Glycyrrhiza glabra* used in this study were collected in Yili, Xinjiang Province, China and authenticated by Dr Ming-xi JIANG, Wuhan Institute of Botany, Chinese Academy of Sciences. The extraction and

identification of ISL are identical with our description in a previous study^[16]. ISL was finally suspended in a 0.5% carboxymethylcellulose (CMC)-saline solution. Creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH) assay kits were from Jian Cheng Biology Research Institute (Nanjing, China). The antibodies for MT-1, MT-2, rabbit anti-JAK 2, anti-STAT 1, anti-STAT 3, anti-ERK 1, anti-ERK 2, and anti-Akt polyclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Phospho-JAK 2, STAT 1, STAT 3, ERK 1, ERK 2, and Akt were from New England Biolabs (Beverly, MA, USA). RNA polymerase chain reaction (PCR) kit (AMV) was purchased from TaKaRa Biotechnology (Dalian, China). All other chemicals were reagents of molecular biology grade obtained from standard commercial sources. AG490 was purchased from Calbiochem-Novabiochem (La Jolla, CA, USA).

Animals and treatment Male Sprague-Dawley rats (Grade II, Certificate No 2004-0004) weighing 250–300 g were purchased from the Experimental Animal Center of Wuhan University. The rats were maintained under standard laboratory conditions at 25±2 °C, relative humidity 50%±15% and normal photo period (12-h dark/12-h light). The animals were fed normal diet and water *ad libitum*. All study protocols were approved by internationally accepted principles and the Guidelines for the Care and Use of Wuhan University, Wuhan, China. Rats were randomly divided into six groups, each consisting of ten animals, (i), sham group; (ii), vehicle group (0.5% sodium CMC+MI/R); (iii), ISL-administration groups (MI/R+ISL), in which the rats were subdivided into 5, 10, and 20 mg/kg groups; and (iv), AG490 group (MI/R+ISL 20 mg/kg+AG490 1 mg/kg). On d 7, 1 h after the above treatments, the rats were subjected to the following evaluation tests.

Surgical preparation Rats of all experimental groups were anaesthetized intraperitoneally with pentobarbitone sodium (60 mg/kg). The neck was opened with a ventral midline incision, and a tracheotomy was performed, the rats were ventilated with room air from a positive pressure ventilator (Crompton Parkinson, Doncaster, Yorkshire, UK). The right carotid artery was cannulated with a polyethylene tube to obtain the blood and extract the serum used for assaying the activities of LDH and CPK at the end of surgery protocol. A left thoracotomy was performed at the fifth intercostals space and the pericardium was opened to expose the heart. The left arterial descending coronary artery (LAD) was ligated 2 mm from its origin by a 5–0 silk suture with a traumatic needle and ends of this ligature were passed through a small vinyl tube to form a snare. The thoracic cavity was covered with saline-soaked gauze to prevent the heart from drying. The animals were allowed to stabilize for 10 min

before LAD ligation. Myocardial ischemia was induced by one stage occlusion of the LAD by pressing the polyethylene tubing against the ventricular wall and then fixing it in place by clamping the vinyl tube with a hemostat. Electrocardiographic leads were attached subcutaneous electrodes to monitor limb lead II. The animals then underwent 15 min of ischemia, confirmed visually *in situ* by the appearance of regional epicardial cyanosis and ST-segment elevation. The myocardium was reperfused by releasing the snare gently for a period of 2 h^[17]. A lead II electrocardiogram was monitored throughout the study. At the end of reperfusion period, rats were killed for biochemical studies and other analysis.

Evaluation of myocardial infarct size The frozen heart tissue samples were cut into 2-mm transverse slices. The slices were incubated in 1% triphenyl tetrazolium chloride (TTC) in pH 7.4 buffer at 37 °C for 20 min. TTC stains living tissue a deep red color while necrotic tissue is TTC negative and is tan in color. The infarct and risk zone considered to be the area lacking fluorescence under UV light was traced.

Determination of arrhythmias Before and during ischemia and reperfusion periods, ECG changes were recorded. The incidences and duration of ventricular premature beats (VPB), ventricular tachyarrhythmia (VT), and ventricular fibrillation (VF) in surviving animals were determined.

Measurement of plasma LDH and CPK activities The blood samples drawn from the carotid artery at the end of MI/R period were collected in heparinized tubes. These samples were kept at 4 °C until they were centrifuged at 2000×g for 15 min. The plasma was recovered and aliquots were used for determination of LDH and CPK activities with commercial kits.

Reverse transcription PCR analysis Total RNA and then cDNA were prepared from rats myocardial tissues using TRIzol RNA extraction and RT-PCR kits. cDNA synthesis, semi-quantitative RT-PCR for MT-1, MT-2, cyclooxygenase-2 (COX-2), iNOS and GAPDH mRNA, and the analysis of the results obtained were performed as described in a previous study^[18]. The sense and anti-sense primer sequences used were: MT-1: 5'-GGTCTTCTCTGTTGGGACA-3', 5'-GCTGGGTTGGGTTGGTCCGATACTA-3'; MT-2: 5'-TAGATGGATCCTGCTCCTGC-3', 5'-CACTTGTAGGAAGCCCTCTT-3'; COX-2: 5'-CCATGTCAAAACCGTGGTGAATG-3', 5'-AGTGAGTTGGGCAGTCATCAG-3'; iNOS: 5'-GTGAGGATCAA-AACTGGGG-3', 5'-ACCTGCAGGTTGGACCACTG-3'; GAPDH: 5'-CCTCTATGCCAACACAGT-3', 5'-AGCCACC-AATCCACACAG-3'. These primers set yielded PCR products of 145, 53, 374, and 200 bp for MT-1, MT-2, and GAPDH, respectively. PCR (26 cycles for MT-1, MT-2 cDNA, 35 cycles for COX-2 cDNA, 32 cycles for iNOS cDNA, and 23 cycles

for GAPDH cDNA) was performed using the GeneAmp PCR System 2400 (Perkin Elmer Life and Analytical Sciences, Boston, MA, USA). The PCR products were electrophoresed through a 1.5% agarose gel and visualized by ethidium bromide staining and UV irradiation.

Western blot analysis Left ventricles from the hearts were homogenized in a buffer containing 25 mmol/L Tris-HCl, 25 mmol/L NaCl, 1mmol/L orthovanadate, 10 mmol/L pyrophosphate, 10 mmol/L okadaic acid, 0.5 mmol/L EGTA, and 1 mmol/L PMSF. Protein (100 µg) of each heart homogenate was incubated with 1 µg of antibody against MT-1, MT-2, JAK 2, STAT 1, STAT 3, ERK 1, ERK 2, and Akt for 1 h at 4 °C. The immune complexes were precipitated with protein A Sepharose, immunoprecipitates separated by SDS-PAGE and immobilized on polyvinylidene difluoride membrane. The membrane was immunoblotted with PY20 to evaluate the phosphorylation of JAK 2, STAT 1, STAT 3, ERK 1, ERK 2, and Akt. The membrane was stripped and reblotted with specific antibodies against MT-1, MT-2, JAK 2, STAT 1, STAT 3, ERK 1, ERK 2, and Akt. The resulting blots were digitized and subjected to densitometric scanning using a standard NIH image program.

Statistic analysis Results of all the above estimations have been indicated in terms of mean±SD. The difference between means was analyzed by ANOVA test. Minimum level of significance was fixed at $P<0.05$.

Results

Effects of ISL on myocardial infarction The myocardial infarction induced by ischemia-reperfusion was measured by TTC staining. As compared with the vehicle group, the percentage of myocardial infarct size was obviously decreased in the ISL 20 mg/kg group from 50.91%±1.90% to 20.45%±1.03% ($P<0.01$) (Table 1). Pretreatment with tyrphostin AG490 significantly blocked the anti-infarct effect of ISL.

Effects of ISL on ischemia-reperfusion arrhythmias There was no significant difference between vehicles and rats treated with ISL with respect to heart rate (HR) immediately prior to coronary occlusion. These were 435±6 and 423±8 beats/min. After coronary artery occlusion, all animals exhibited cardiac arrhythmias, which occurred as VPB, VT, and VF. In some animals, atrioventricular (A-V) block also occurred. On subsequent reperfusion, arrhythmia severity was much less marked in those rats given ISL (Table 2). VPB developed in 100% of vehicles versus 62.5%, 42.8%, and 25% ($P<0.05$) of ISL 5, 10, and 20 mg/kg treated animals, respectively. The incidences of VF that occurred in 83% of the vehicle group were decreased to 0 in the ISL 20 mg/kg

Table 1. Effects of ISL on myocardial infarction in MI/R rats. $n=10$. Mean \pm SD. ^b $P<0.05$ vs sham group. ^c $P<0.05$ vs vehicle group. ⁱ $P<0.01$ vs ISL 20 mg/kg group.

Group	Dose/mg·kg ⁻¹	Infarct size/%
Sham	0	–
Vehicle	0	50.91 \pm 1.90 ^b
ISL	5	36.65 \pm 0.54 ^c
	10	30.72 \pm 0.76 ^c
	20	20.45 \pm 1.03 ^c
AG490	1	33.20 \pm 0.50 ⁱ

Vehicle, MI/R+0.5% sodium CMC; ISL: isoliquiritigenin (5, 10, and 20 mg/kg, ig); AG490, MI/R+ISL 20 mg/kg+AG490 1 mg/kg.

Table 2. Effects of ISL on arrhythmias in MI/R rats. $n=10$. Mean \pm SD. ^b $P<0.05$ vs sham group. ^c $P<0.05$, ^f $P<0.01$ vs vehicle group. ⁱ $P<0.01$ vs ISL 20 mg/kg group.

Group	Dose /mg·kg ⁻¹	Incidence/%			Duration /min
		VPB	VT	VF	
Sham	0	–	–	–	–
Vehicle	0	100.0	83.3	80.0	3.14 \pm 1.05 ^b
ISL	5	60.0	60.0	50.0	2.33 \pm 0.98 ^c
	10	40.0	20.0	20.0	1.90 \pm 1.00 ^c
	20	20.0	30.0	0.0	0.37 \pm 0.06 ^f
AG490	1	50.0	40.0	30.0	2.10 \pm 0.07 ⁱ

Vehicle, MI/R+0.5% sodium CMC; ISL, isoliquiritigenin (5, 10, and 20 mg/kg, ig); AG490, MI/R+ISL 20 mg/kg+AG490 1 mg/kg. VPB: ventricular premature beats; VT: ventricular tachycardia; VF: ventricular fibrillation.

group. Also, ISL markedly shortened the duration of ventricular arrhythmias.

Effects of ISL on plasma LDH and CPK activities The biochemical indicators of myocardial damage were evaluated in the MI/R period. When plasma LDH and CPK activities in rats treated with ISL, the results shown in Table 3 were obtained. Compared to the vehicle group, pretreatment of rats with ISL (5, 10, and 20 mg/kg, ig) markedly decreased the LDH and CPK activities by 22%, 34%, 38% and 35%, 52%, 58%. The decreases of LDH and CPK releases partly disappeared with the inhibition of AG490.

Effects of ISL on MT-1, MT-2 mRNA and protein expressions This study investigated the effect of ISL on the regulation of MT expressions in MI/R rat model. RT-PCR demonstrated specific amplifications of MT, COX-2, and iNOS cDNA. MT-1 and MT-2 mRNA were upregulated by ISL in a

Table 3. Effects of ISL on plasma LDH and CPK activities in MI/R rats. $n=10$. Mean \pm SD. ^c $P<0.01$ vs sham group. ^e $P<0.05$, ^f $P<0.01$ vs vehicle group. ^h $P<0.05$ vs ISL 20 mg/kg group.

Group	Dose /mg·kg ⁻¹	LDH /U·mL ⁻¹	CPK /U·mL ⁻¹
Sham	0	0.11 \pm 0.05	1.17 \pm 0.27
Vehicle	0	1.36 \pm 0.18 ^c	4.12 \pm 1.09 ^c
ISL	5	1.03 \pm 0.19 ^e	2.48 \pm 0.17 ^f
	10	0.89 \pm 0.11 ^f	2.06 \pm 0.07 ^f
	20	0.81 \pm 0.05 ^f	1.94 \pm 0.48 ^f
AG490	1	1.18 \pm 0.15 ^h	2.12 \pm 0.11 ^h

Vehicle, MI/R+0.5% sodium CMC; ISL, isoliquiritigenin (5, 10, and 20 mg/kg, ig); AG490, MI/R+ISL 20 mg/kg+AG490 1 mg/kg. LDH: lactate dehydrogenase; CPK: creatinine phosphokinase; MI/R: myocardial ischemia-reperfusion.

dose-dependent manner. There were no significant differences of COX-2 or iNOS mRNA levels among ISL-treated groups when compared to the vehicle group (Figure 1). With the dose of ISL increased, the protein expressions of MT-1, MT-2 were obviously increased. This result indicated that MT expressions were upregulated by ISL in myocardium through transcriptional activation. In contrast, the increased MT expression in the ISL 20 mg/kg group was blocked by tyrphostin AG490.

Effects of ISL on JAK /STAT phosphorylations To determine the underlying molecular mechanism of the JAK 2 inhibitor-induced decrease of MT expression, the phosphorylation of JAK 2, STAT 1, STAT 3, ERKs, and Akt were examined by Western blot analysis. The results suggested that pretreatment with AG490 30 min before the coronary artery ligation combined with ISL 20 mg/kg significantly inhibited the phosphorylation of STAT 3. STAT 1 was weakly phosphorylated in MI/R rats, however, it was not obviously affected by AG490 treatment (Figure 2). Other signal transducing molecules downstream of gp130, such as ERK and Akt were also analysed with Western blotting by using phosphor-specific antibodies. As shown in Figure 2, neither Akt nor ERK was activated in MI/R rats of ISL-treated groups, indicating that JAK 2/STAT 3 may be exclusively activated.

Discussion

In the present study, we demonstrated that ISL induced the upregulation of MT expression. As expected, ISL also resulted in cardioprotection as evidenced by attenuated ventricular arrhythmias, reduced infarct size, and decreased the releases of LDH and CPK. The ISL treated MI/R rats

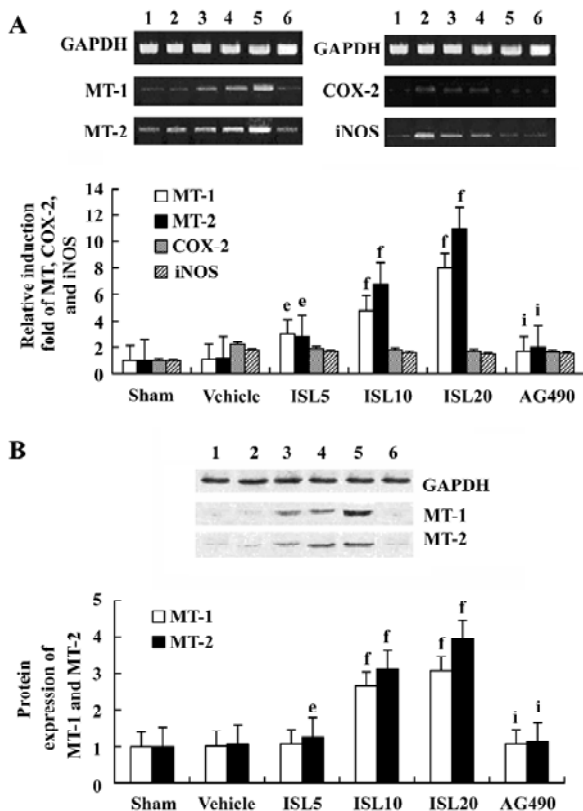


Figure 1. Effects of isliquiritigenin (ISL) on metallothionein (MT), COX-2, and iNOS mRNA and MT protein expression in MI/R rats. (A) MT, COX-2, and iNOS mRNA expressions were analyzed by reverse transcription-polymerase chain reaction (RT-PCR). The bar chart below the blot shows the semiquantitative analysis of the MT protein expression (normal group=1). (B) MT-1 and MT-2 protein levels were determined by Western blotting. GAPDH was analyzed as a sample loading control. The bar charts shows the semiquantitative analysis of expression of MT. Lane 1, Sham; Lane 2, Vehicle (MI/R+0.5% sodium CMC); Lane 3, 4, and 5, ISL 5, 10, and 20 mg/kg; Lane 6, AG490 (MI/R+ISL 20 mg/kg+AG490 1 mg/kg). *n*=10. Mean±SD. ^e*P*<0.05, ^f*P*<0.01 vs vehicle group. ⁱ*P*<0.01 vs ISL 20 mg/kg group.

revealed increased phosphorylations of JAK2/STAT3 but not of ERKs and Akt pathway. Inhibition of JAK2 kinase with tyrphostin, AG490 resulted in the inhibition of STAT3 phosphorylation, decreased the expressions of MT-1 and MT-2, and abrogated the cardioprotection effects of ISL.

Myocardial reperfusion through thrombolysis, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting is standard treatment in acute myocardial infarction. However, these therapies initiate a second phase of myocardial injury either by acceleration of detrimental processes initiated during ischemia or by inducing additional pathological processes following reperfusion^[19,20]. Our results indicated that in MI/R model group, serious

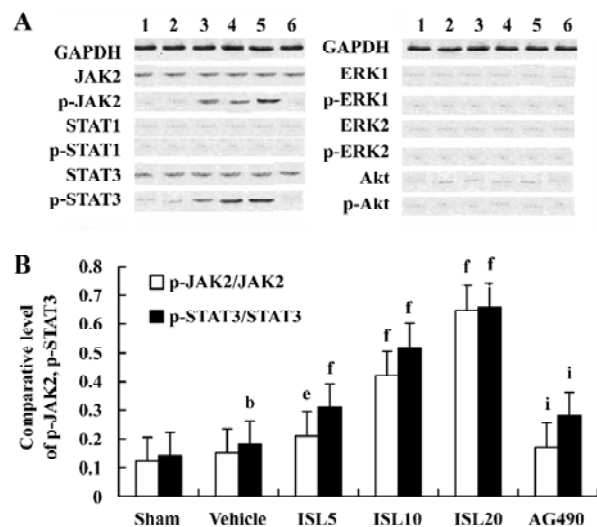


Figure 2. Effects of ISL on phosphor-janus kinase 2 (JAK 2) and phosphor-signal transducers, activators of transcription 1, 3 (STAT 1, 3), extracellular-signal regulated kinase (ERK), and Akt expressions in MI/R rats. (A) Phospho-JAK 2 and phosphor-STAT 3 were up-regulated in ISL groups in a dose-dependent manner, while STAT1 was weakly phosphorylated. Phospho-ERK1, ERK2, and Akt showed no significant changes after treatment with ISL. (B) The bar chart shows the semiquantitative analysis of the protein expression. Lane 1, Sham; Lane 2, Vehicle (MI/R+0.5% sodium CMC); Lane 3, 4, and 5, ISL 5, 10, and 20 mg/kg; Lane 6, AG490 (MI/R+ISL 20 mg/kg+AG490 1 mg/kg). *n*=10. Mean±SD. ^b*P*<0.05 vs sham group. ^e*P*<0.05, ^f*P*<0.01 vs vehicle group. ⁱ*P*<0.01 vs ISL 20 mg/kg group.

myocardial infarction and ventricular arrhythmias were observed. Besides this, the activities of LDH and CPK in the plasma were much higher than those in the sham group.

Increasing clinical and experimental data have provided evidence that ischemic cell injury is mediated by ROS^[21]. The role of oxygen free radicals in the pathophysiology of ischemia-reperfusion injury is supported by the increased formation of lipid peroxides and other toxic products following such as injury^[22]. The interaction of ROS with cell membrane lipids and essential proteins contributes to myocardial cell damage, leading to inflammatory reactions, irreversible tissue injury, and consequently, to impaired cardiac function^[23].

MT, a low molecular protein rich in *L*-cysteine have been shown to be associated with resistance to oxidative stress in cardiac protection against ischemia-reperfusion damage^[24]. In this study, we found that both the mRNA and protein expressions of MT were upregulated by ISL. These results are in accordance with previous studies on the antioxidative role of ISL.

Originally described as the regulator for cytokine signaling,

JAK/STAT pathway is now recognized as an important membrane-to-nucleus signaling pathway for a variety of stress responses including ischemia, hypoxia, and oxidative stress. In response to ischemic stress, phosphorylation of JAK occurs followed by tyrosine phosphorylation and dimerization of cytosolic STAT monomers. The STAT dimers readily translocate to the nucleus, bind to the promoter regions of the DNA and regulate transcription of genes^[25]. The JAK/STAT pathway, especially the JAK2/STAT3-dependent pathway, may have a cytoprotective effect on stretched myocardium in MI/R hearts^[4]. Treatment with JAK2 inhibitor reduced the phosphorylation of STAT1, 3 in MI hearts^[26]. STAT3 also showed a transducer protective signal against oxidative stress and doxorubicin-induced cardiomyopathy^[27]. The current study showed that ISL increased JAK2/STAT3 phosphorylation levels and led to upregulation of MT levels in MI/R rat. These results are consistent with previously published reports that state that specific activation of STAT3 transducers antioxidative signals through MT and confer resistance against ROS stress in the heart.

It has been reported that COX-2 and iNOS are the downstream targets of the JAK/STAT pathway and play an important role in ischemia-reperfusion injury^[4,28]. Thus, we examined the mRNA expression of COX-2 and iNOS by RT-PCR and found no significant difference of iNOS and COX-2 mRNA levels in ISL-treated groups when compared to the vehicle group. These findings suggest that COX-2 and iNOS is may not be involved in ISL-induced cardioprotection.

In summary, we demonstrated that ISL, a flavonoid, strongly reduced myocardial infarct size and prevented reperfusion-induced arrhythmias in the model of MI/R rats *in vivo*. It also appears that activation of JAK2/STAT3 and subsequent upregulation of MT is instrumental, at least in part, in ISL-mediated cardioprotection.

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