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Sulfur dioxide restores calcium homeostasis disturbance in rat with isoproterenol-induced myocardial injury

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Summary. Backgrounds: sulfur dioxide (SO_2) could relieve isoproterenol (ISO)-induced myocardial injury, while the mechanism is unclear. This study aims to explore whether the protective effect of SO_2 on ISO-induced myocardial injury was mediated by the restoration of calcium homeostasis disturbance in cardiomyocyte. Methods and results: Rats were randomly divided into four groups: ISO group, ISO+SO_2 group, control group and SO_2 group. Content of Ca^{2+} in H9c2 cells was assayed using confocal microscope, and cardiac function parameters were measured by echocardiography. Plasma biochemical values and myocardial ultra-structure changes were measured. Meanwhile, the activity, protein and gene levels of sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), and protein and phosphorylation of phospholamban (PLN) were detected. We found SO_2 derivatives could restore the decreased cardiac function, the abnormal lactate dehydrogenase, creatine kinase, alpha-hydroxybutyrate dehydrogenase, potassium, calcium, blood urea nitrogen and the damaged myocardial ultra-structure in rats, and regulate the increased Ca^{2+} content in H9c2 induced by ISO. In addition, compared with ISO group, the decreased activities, protein and mRNA level of SERCA, as well as the decreased protein phosphorylation level of PLN in myocardial tissues were increased in ISO+SO_2 group. Conclusion: SO_2 derivatives might relieve calcium overload in association with the upregulating expression of SERCA and p-PLN/PLN by myocardial tissues in rats with ISO-induced myocardial injury.

Key words: Sulfur dioxide, Isoproterenol, Myocardial injury, Calcium homeostasis

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Introduction

Myocardial injury is a common pathophysiologic process of many cardiovascular diseases, such as myocarditis (Kamal et al., 2011), myocardial ischemia (Zhang et al., 2009a,b; Wang et al., 2011) and myocardial infarction (Angeli et al., 2009; Wei-hua et al., 2011). However, its mechanisms are very complicated, mainly including oxidative stress (Liang et al., 2011), endoplasmic reticulum stress (Wang et al., 2011), and calcium overload (Zeitz et al., 2002). Calcium overload, as a result of deregulation of calcium homeostasis, has been reported to be responsible for the myocardial injury of ischemia-reperfusion (Kim et al.,

Abbreviations: α-HBDH, alpha-hydroxybutyrate dehydrogenase; LDH, lactate dehydrogenase; BSA, bovine serum albumin; LVAWd, diastolic left ventricular anterior wall thickness; BUN, blood urea nitrogen; LVAWs, systolic left ventricular anterior wall thickness; Ca, calcium; LVPWd, diastolic left ventricular posterior wall thickness; CK, creatine kinase; LVPWs, systolic left ventricular posterior wall thickness; Cr, creatinine; Mg, magnesium; DMEM, Dulbecco's modified Eagle's medium; Na, Sodium; EF, ejection fraction PLN, phospholamban; FBS, fetal bovine serum; p-PLN, phosphorylation of phospholamban; FS, fractional shortening; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SO_2, sulfur dioxide; HCO_3^-, bicarbonate; SR, sarcoplasmic reticulum; ISO, isoproterenol; TEM, transmission electron microscope; K, potassium

2010) and heart failure (Zhang et al., 2009a,b). The injury could be improved via inhibition of calcium overload. Thus, it is attracting more and more researchers to consider calcium homeostasis as a key therapy target.

In the model of isoproterenol (ISO)-induced myocardial injury, we found that the aspartate amino transferase/sulfur dioxide pathway was down-regulated, and the administration of SO_2 could improve the cardiac functions with the up-regulated endogenous SO_2 level. We also demonstrated that oxidative stress and endoplasmic reticulum stress were responsible for the protective effect of SO_2 on myocardial injury (Chen et al., 2011, 2012; Liang et al., 2011).

Nie and Meng (2006) found that SO_2 derivatives could regulate the voltage-dependent L-type calcium current (ICa,L) in isolated rat ventricular myocytes. Zhang and Meng (2009) reported the vasodilator effect of SO_2 on isolated aortic rings of rats, and they suggested the mechanism might be related to the L-type calcium channel. Our previous studies also demonstrated SO_2 derivatives could inhibit the L-type calcium channel on isolated aortic rings of rats (Du et al., 2008a,b) and depress the L-type calcium current in isolated rat ventricular cardiomyocytes (Zhang et al., 2011). However, whether SO_2 provides a protective effect in rats with ISO-induced myocardial injury via regulation of calcium homeostasis is unclear. Therefore, in the present study, we attempted to investigate whether the regulatory effect of SO_2 on myocardial injury caused by ISO was related to calcium homeostasis.

Materials and methods

Reagents

Isoproterenol hydrochloride and Na_2SO_3/$NaHSO_3$ (SO_2 derivatives) were purchased from Sigma (St. Louis, MO, USA). H9c2 cells were obtained from American Type Culture Collection (Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trizol reagent, PLN and p-PLN were obtained from Invitrogen (Carlsbad, CA, USA). SERCA was from ABR (USA). Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) and secondary antibodies were obtained from Santa Cruz (Santa Cruz, CA, USA). $Oligod(T)_{15}$ primer, M-MLV reverse transcriptase and dNTP were from Promega (Madison, WI, USA). All other chemicals were of analytical grade and purchased from Beijing Chemical Reagents (Beijing, China).

Preparation of H9c2 cells cell

H9c2 rat embryonic cardiac myoblasts were cultured in high glucose (4.5 g/L) DMEM supplemented with 10% FBS, 4 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were routinely grown to subconfluency (>90% by visual estimate) in 75 cm^2 flasks at 37°C in a humidified atmosphere of 5% CO_2 and 95% air prior to passage and seeding for experiments.

Measurement of the myocardial intracellular free Ca^{2+} concentration

H9c2 cells were incubated with the fluo-3 AM (a final concentration of 10 μmol/ L) which was mixed with Pluronic-F127 (a final concentration of 0.05%) for 30 min at 37°C in the dark. After incubation, cells were washed with D'-Hanks solution with 0.2% bovine serum albumin (BSA) twice and then D'-Hanks solution without BSA once to remove remaining dye. Then they were placed under the TCS SP2 laser scanning confocal microscope (LEICA Microsystems, Mannheim, Germany) to observe the fluorescence intension of intracellular free Ca^{2+}. The intracellular Fluo-3 was excited with light at 488 nm, and the emitted fluorescence was detected at 530 nm. Fluorescence images were acquired at 10 s intervals.

To observe the effects of SO_2 derivatives on ISO-induced increase of the intracellular free Ca^{2+} concentrations, ISO (a final concentration of 50 μmol/ L) was added after the basal level was observed for 2 min and SO_2 derivatives (a final concentration of 20 μmol/L) was added into the solution 2 min after ISO. The activity of H9c2 cell was examined by KCL (10 mol/L). The total images were collected and analysed by LEICA imaging software (LEICA Microsystems, Mannheim, Germany).

Preparation of the animal model

The study protocol was approved by the Animal Research Committee of Peking University (Beijing, China). Thirty two male Wistar rats (230-260 g) purchased from Animal Center of Chinese Academy of Medical Sciences were randomly divided into 4 groups depending on treatment: ISO group where the rats were administered with isoproterenol hydrochloride at 20 $mg.kg^{-1}.d^{-1}$ (Wang et al., 2010) via subcutaneous injection, ISO+SO_2 group where the rats were administered with isoproterenol hydrochloride at 20 $mg.kg^{-1}.d^{-1}$ via subcutaneous injection and SO_2 derivatives Na_2SO_3/$NaHSO_3$ at 85 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ (Sun et al., 2010) via intraperitoneal injection, the control group where the rats were administered with saline via subcutaneous injection, and the SO_2 group where the rats were administered with Na_2SO_3/$NaHSO_3$ at 85 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ via intraperitoneal injection. All rats were administered with the different drugs for 7 days.

Echocardiographic study

On the 7th day of the study, 24 h after the last administration of drugs, the rats were anesthetized with a mixture of 3 % isoflurane and oxygen. The chest of rats was shaved with electric clippers and electrodes were attached to their extremities for electrocardiogram recording. The hearts were imaged in a shallow left

lateral decubitus position, and a 17.5 Hz probe of two-dimensional echocardiography scanner was used to detect the ejection fraction (EF), fractional shortening (FS), the systolic left ventricular anterior wall thickness (LVAWs), the diastolic left ventricular anterior wall thickness (LVAWd), the systolic left ventricular posterior wall thickness (LVPWs) and the diastolic left ventricular posterior wall thickness (LVPWd). All data were averaged from measurements of three consecutive cardiac cycles.

Determination of biochemical values in plasma of rats

At the end of echocardiographic study, rats were anesthetized with 12% urethane (10 ml/kg, intraperitoneally) and plasma was collected from the abdominal aorta and stored at -70°C. Lactate dehydrogenase (LDH), creatine kinase (CK), alpha-hydroxybutyrate dehydrogenase (α-HBDH), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), bicarbonate (HCO_3^-), creatinine (Cr), and blood urea nitrogen (BUN) levels in plasma of rats were detected by an automatic biochemistry analyzer (Hitachi 7600, Tokyo, Japan).

Determination of ultra-structural changes in myocardial tissues using transmission electron microscope (TEM)

Ultra-structural changes were examined in the left ventricular apex with TEM. Fresh myocardial tissues were cut into pieces (1 mm^3), doubly fixed with 3% glutaraldehyde and 1% osmium tetroxide, dehydrated with graded acetone, and embedded with Epon812. The prepared tissue was cut into ultrathin sections with a Leica UCT slicer and stained with both uranyl acetate and lead citrate. Finally, sections were observed under transmission electron microscope (JEM 1230, TEM, Japan) and photographed.

Determination of Ca^{2+}-ATPase (SERCA) activity in myocardial tissues

The rate of ATP hydrolysis was measured using the colorimetric method as described previously (Wang et al., 2007) and absorbance was measured at 636 nm with a ultraviolet-visible spectrophotometer (UV2100, Shimadzu, Japan). Ca^{2+}-ATPase activity was determined by subtracting Mg^{2+}-dependent activity from the activity measured in the presence of both Mg^{2+} and Ca^{2+}, and Mg^{2+}-dependent activity was measured in a medium without Ca^{2+}.

Measurement of protein expression of SERCA, PLN and p-PLN in myocardium using Western blotting

Myocardia of rats were lysed, and total protein samples (50 μg) were separated on a 10% sodium dodecylpolyacrylamide gel electrophoresis for 2 h at 100 V and then transferred to a nitrocellulose membrane (Amersham, USA) for 2 hours at 200 mA. After blocking, the membranes were incubated with 5 % non-fat milk for 1 h at room temperature and then incubated with primary antibodies overnight at 4°C: SERCA (dilution of 1:1000), PLN (dilution of 2000), p-PLN (dilution of 1000) and GAPDH (dilution of 1:5000). On the second day, anti-rabbit or anti-mouse IgG antibodies were used at a dilution of 1:10 000 for 1 h at room temperature. Immunoreactions of the membranes were visualized by electrochemiluminescence and exposed to X-ray film (Kodak Scientific). The amount of the bands was quantified by densitometry using image software, and the expression level was calculated as the intensity ratio of protein to that of GAPDH.

Measurement of the gene expression of SERCA in myocardia using quantitative real-time polymerase chain reaction (real-time PCR)

Total RNA was extracted from cardiac tissues using trizol reagent, according to the manufacturer's instructions. First-strand cDNA was synthesized with

Table 1. The sequence of the primers and probes of mRNA.

Target genes	Primers and probes	Sequence	Size of product (bp)
SERCA	Forward primer	5'-CGTTGAGTTCCTGCAGTCCTT	91
	Reverse primer	5'-CCCGATTTCCGACTTCTTCA	
	TaqMan probe	5'-ATGAGATCACAGCTATGACTGGTGATG	
ß-actin	Forward primer	5'-ACCCGCGAGTACAACCTTCTT	80
	Reverse primer	5'-TATCGTCATCCATGGCGAACT	
	TaqMan probe	5'-CCTCCGTCGCCGGTCCACAC	

Table 2. Echocardiographic parameters in different groups (mean±SD).

Group	N	EF (%)	FS (%)	LVAWs (mm)	LVAWd (mm)	LVPWs (mm)	LVPWd (mm)
control group	8	75.57±4.62	45.71±4.19	2.68±0.31	1.75±0.40	2.54±0.21	1.65±0.16
ISO group	7	59.17±4.27**	32.41±2.90**	3.63±0.67*	2.75±0.58**	3.14±0.36**	2.33±0.39**
ISO+SO_2 group	8	75.56±6.28##	46.19±6.15##	3.30±0.40	1.97±0.17##	2.79±0.23#	1.87±0.19##

EF, ejection fraction; FS, fractional shortening; LVAWs, systolic left ventricular anterior wall thickness; LVAWd, diastolic left ventricular anterior wall thickness; LVPWs, systolic left ventricular posterior wall thickness; LVPWd, diastolic left ventricular posterior wall thickness. *: $p<0.05$ vs control group, **: $p<0.05$ vs control group, #: $p<0.05$ vs ISO group, ##: $p<0.01$ vs ISO group.

oligod(T)15 primer and M-MLV reverse transcriptase. The primers and probes were designed with the Primer Express 3.0 software (Applied Biosystems, Foster City, CA, USA) and synthesized by SBS Company (Beijing, China). The primer sequences and probes are shown in Table 1.

Quantitative real-time PCR was carried out with an ABI PRISM 7300 instrument (Applied Biosystems). And the PCR condition was pre-denatured at 95°C for 10 min, denatured at 95°C for 15 s, and renatured at 60°C for 1 min for 40 cycles. The amount of ß-actin cDNA was used as a control.

Data analysis

All data were performed in SPSS 13.0 statistical analysis software and presented as means ± SD. Differences among groups were assessed using One-way ANOVA followed by a *post hoc* analysis (least-square difference test). P<0.05 was considered statistically significant.

Results

The myocardial intracellular free Ca^{2+} concentration in H9c2 cells

Compared with the basal level, the fluorescence intensity of intracellular free Ca^{2+} was enhanced after adding ISO. However, the administration of SO_2 derivatives could weaken the fluorescence intensity of intracellular free Ca^{2+}. This suggested that SO_2 could relieve ISO-induced increase in the intracellular free Ca^{2+} concentration (Fig. 1).

Echocardiographic parameters

Compared with those of the control group, EF and FS were significantly decreased (both $P<0.01$) while LVAWs, LVAWd, LVPWs and LVPWd significantly increased ($P<0.05$, $P<0.01$, $P<0.01$ and $P<0.01$, respectively) in rats of ISO group. After the administration of SO_2 derivatives, EF and FS were significantly increased (both $P<0.01$) while LVAWd, LVPWs and LVPWd significantly decreased ($P<0.01$, $P<0.05$ and $P<0.01$, respectively). However, there was no statistical difference in LVAWs between ISO group and ISO+SO_2 group (Table 2).

Biochemical values in plasma of rats

The plasma levels of LDH, CK and α-HBDH increased significantly in ISO-treated rats compared with

Table 3. Myocardial enzymes LDH, CK and α-HBDH levels in plasma of rats.

group	LDH (U/L)	CK (U/L)	α-HBDH (U/L)
control group (n=8)	127.6±19.0	264.1±31.8	63.1±4.0
ISO group (n=7)	192.7±29.3**	401.3±51.7**	88.0±13.5*
ISO+SO_2 group (n=8)	148.1±25.7##	317.3±41.2##	70.1±10.7#
SO_2 group (n=8)	133.5±34.9	270.6±51.7	65.3±8.2

LDH, lactate dehydrogenase; CK, creatine kinase; α-HBDH, alpha-hydroxybutyrate dehydrogenase. **: $P<0.01$ vs control group, ##: $P<0.01$ vs ISO group, #: $P<0.05$ vs ISO group.

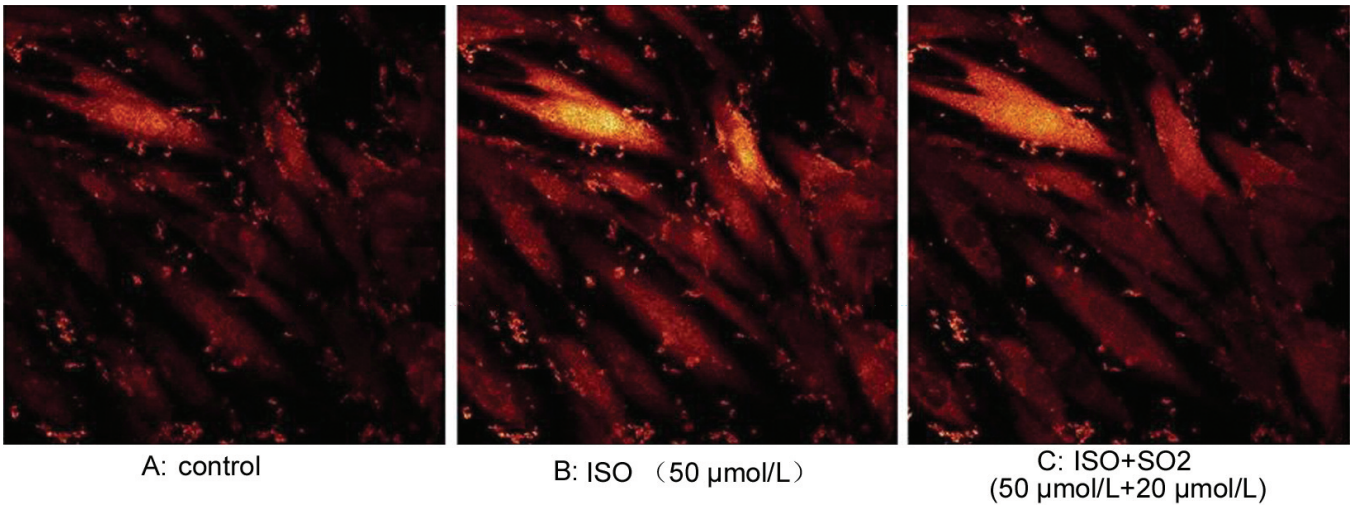

Fig. 1. Fluorescence images of $[Ca^{2+}]_i$ changes in H9c2 by confocal microscopy. **A.** The control$[Ca^{2+}]_i$ in basal level. **B.** The fluorescence images of $[Ca^{2+}]_i$ in the presence of 50 μmol / L ISO at 2 min. **C.** The fluorescence images of $[Ca^{2+}]_i$ in the presence of 20 μmol / L SO_2 at 2 min. ISO, isoproterenol; SO_2, sulfur dioxide.

those of control rats, which indicated that myocardiocytes were damaged. K, Ca, and BUN in plasma also increased in ISO group compared with the control group, while sodium, magnesium, bicarbonate and creatinine in plasma showed no statistical differences between the ISO group and the control group. SO_2 administration, however, decreased the plasma level of LDH, CK, α-HBDH, K, Ca, and BUN. There were no statistically significant differences between the control group and the SO_2 group, which suggested that $Na_2SO_3/NaHSO_3$ at 85 $mg.kg^{-1}.d^{-1}$ for 7 days did not cause electrolyte imbalance and nephrotoxicity. On the contrary, $Na_2SO_3/NaHSO_3$ was able to restore ISO-induced electrolyte imbalance and nephrotoxicity (Tables 3, 4).

Ultra-structural changes in myocardial tissues

Under TEM, we observed the ultra-structure changes in myocardial tissues. In the control rats, myocardial fibers were ordered and sarcoplasmic reticulum (SR) and T tubular structure of mitochondria were clearly observed (Fig. 2a), while in ISO-treated rats, we observed that the myocardial fiber structure was disordered, large vacuoles were present within the cytoplasm, mitochondria were swollen and the crests were broken or dissolved. SR and T tubules were in a high degree of expansion, the lamellar body could be seen, and there were fewer glycogen particles (Fig. 2b). However, with the administration of SO_2 derivatives, myocardial fibers and the structure of most mitochondria were clear, although SR and T tubules of some mitochondria showed moderate expansion (Fig. 2c).

Activity, protein and gene expression of SERCA in myocardial tissues

Compared with control group rats, SERCA activities and protein and gene expression in the myocardium in rats of ISO group were significantly decreased (all $P<0.05$); whereas in ISO+SO_2-treated rats, SERCA activities and protein and gene expression in the myocardium were significantly increased compared with those in rats of ISO group (all $P<0.05$) (Fig. 2A-C).

Protein expression of p-PLN/PLN in myocardial tissues

Compared with the control group rats, the protein ratio of p-PLN/PLN in the myocardial tissues of rats in ISO group showed a significant decrease ($P<0.05$),

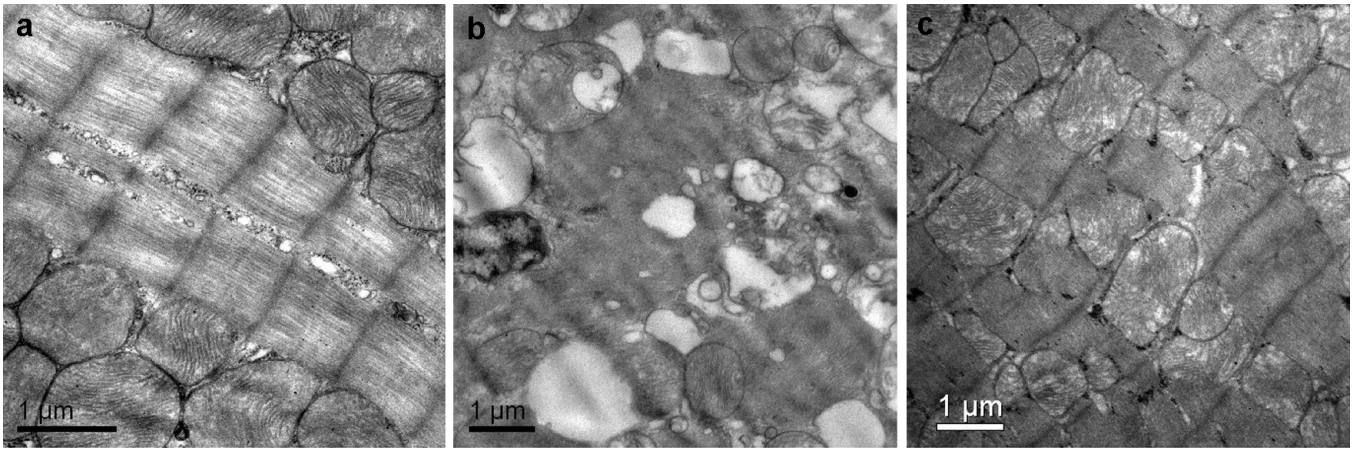

Fig. 2. Ultrastructural changes of myocardial tissues under transmission electron microscope. **a.** Photograph from rat in control group. **b.** Photograph from rat in ISO group. **c.** Photograph from rat in ISO+SO_2 group.

Table 4. Na, K, Ca, Mg, HCO_3^-, Cr and BUN in plasma of rats.

group	Na (mmol/L)	K (mmol/L)	Ca (mmol/L)	Mg (mmol/L)	HCO_3^- (mmol/L)	Cr (μmol/L)	BUN (mmol/L)
control group	141.4±8.0	7.38±0.51	2.11±0.12	1.45±0.19	18.6±3.9	49.5±7.0	5.49±1.10
ISO group	146.4±7.3	9.95±1.69*	2.40±0.09**	1.36±0.18	15.8±1.4	45.0±7.7	7.09±0.73**
ISO+SO_2 group	142.1±6.5	7.53±0.85$^{\#}$	2.17±0.13$^{\#\#}$	1.44±0.12	15.5±1.0	48.3±4.3	6.13±0.76$^{\#}$
SO_2 group	143.9±6.8	7.51±0.56	2.13±0.18	1.45±0.09	18.0±1.8	44.9±7.4	5.56±0.85

Sodium, Na; potassium, K; calcium, Ca; magnesium, Mg; bicarbonate, HCO_3^-; creatinine, Cr; blood urea nitrogen, BUN. *: $P<0.05$ vs control group, **: $P<0.01$ vs control group, $^{\#}$: $P<0.05$ vs ISO group, $^{\#\#}$: $P<0.01$ vs control group.

while compared with the ISO group, the protein ratio of p-PLN/PLN showed a significant increase in the ISO+SO_2-treated rats (P<0.05) (Fig. 2D).

Discussion

Myocardial injury is involved in the pathophysiologic process of many cardiovascular diseases and is responsible for decreased cardiac functions. Therefore, it is important to explore its mechanism and therapeutic modalities. In the present study, we found that the mortality rate was 20.8% in ISO group and 14.3% in ISO+SO_2 group, while 0% in the control group. In the model of ISO-induced myocardial injury, we observed increased LDH, CK and α-HBDH, as well as damaged myocardial ultra-structure. These suggested that myocardial injury was modeled successfully.

Researchers used to consider SO_2 as being harmful to human health (Meng et al., 2004; Curtis et al., 2006), and epidemiological investigations showed that individuals who were often exposed to SO_2 had a high risk of developing cardiovascular diseases (Dippoliti et al., 2003; Rich et al., 2005). However, studies (Ji et al., 1995; Du et al., 2008a,b) demonstrated that SO_2 could be generated endogenously. Also our research on model of Langendorff isolated rat heart (Zhang et al., 2008, 2009a,b) showed that SO_2 derivatives could inhibite $\pm dp/dt_{max}$ and left ventricular pressure in a dose-dependent manner (10^{-6}~10^{-3} mol/L) in isolated rat heart with ischemia and reperfusion. This suggested that SO_2 might be another novel gasotransmitter apart from the NO, CO and H_2S involved in the regulation of cardiac functions (Liu et al., 2010; Wang et al., 2010a,b; Chen et al., 2011, 2012).

Our previous study found that the downregulation of endogenous SO_2/GOT pathway was responsible for the development of myocardial injury induced by ISO, and the administration of SO_2 could improve the cardiac functions with the up-regulated endogenous SO_2 level (Liang et al., 2011). Our present study also found that SO_2 could decrease the increased level of cardiac enzymes and ameliorate ISO-induced myocardial ultra-structure damage. However, the mechanism responsible for the protective effect of SO_2 is unclear.

Calcium homeostasis is important in maintaining the normal structure and function of a somatic cell (Szabó and Salzman, 1996; Zatisev et al., 2001), but some

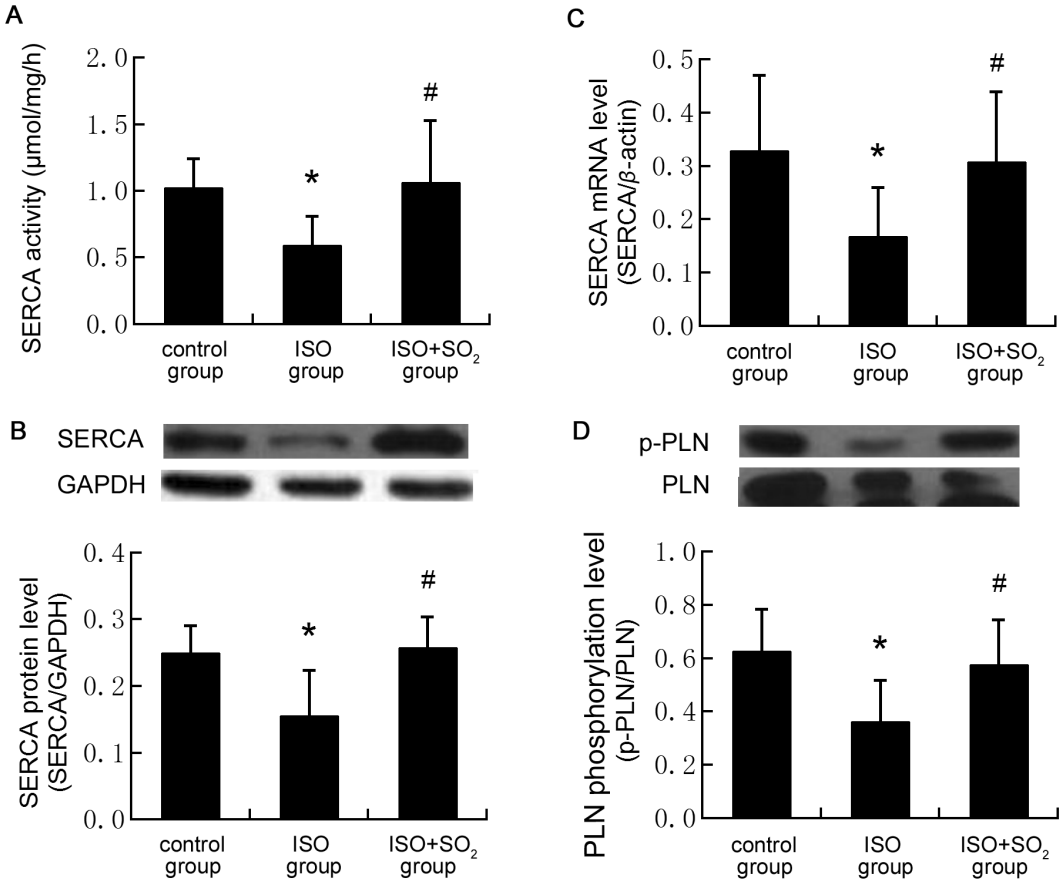

Fig. 3. Activity, protein and mRNA level of SERCA, and phosphorylation level of PLN. ISO, isoproterenol; SO_2, sulfur dioxide. *: p<0.05 vs control group, #: p<0.05 vs ISO group.

harmful factors can cause the dysfunction of the calcium homeostasis system and disorders of calcium distribution, and eventually lead to the increased intracellular calcium concentration, which is calcium overload. Calcium overload could cause damage to the membrane potential of mitochondria (Zatisev et al., 2001) and activate Ca proteinase directly (Szabó and Salzman, 1996), thus leading to the death of cells. SO_2 was reported to inhibit the L-type calcium channel on isolated aortic rings of rats (Du et al., 2008a,b) and depress the L-type calcium current in isolated rat ventricular cardiomyocytes (Zhang et al., 2011), which might be related to the protective effect. Therefore, we planned to explore whether SO_2 played a myocardial protective effect through regulating dysfunction of the calcium homeostasis system. Results showed that SO_2 could restore intracellular calcium overload induced by ISO, demonstrated by the decreased fluorescence intensity of intracellular free Ca^{2+}.

SERCA is recognized as being responsible for intracellular calcium homeostasis, and monomeric PLN binds to and inhibits SERCA, while phosphorylated PLN disrupts this inhibitory complex (MacLennan et al., 1998). Does SO_2 regulate intracellular calcium homeostasis through SERCA and PLN? We examined the expression of SERCA and PLN in myocardial tissues of rats. We found that SERCA activities, protein and mRNA level, and the p-PLN/PLN level were all decreased in rats of ISO group compared with those of control group. However, after treatment with SO_2 derivatives, SERCA activities, protein and mRNA level, and the p-PLN/PLN level were all increased to the level of those of the control group. This indicated that SO_2 could restore intracellular calcium overload induced by ISO, and its mechanism may be related to the fact that SO_2 increased protein phosphorylation level of PLN, thereby enhancing the expression and activity of SERCA. This result has important significance to explain the mechanism for the myocardial protective effect of SO_2.

The concentration of SO_2 in the study was based on the physiological concentration of SO_2 in human and rats. Ji et al. (1995) reported the concentration of total serum sulfite in healthy donors was 4.87±2.49 μmol/L and the reference range for total serum sulfite in normal donors was 0-9.85 μmol/L. Our previous research (Du et al., 2008a,b) showed the concentration of SO_2 in heart of rats was 1.78±0.12 μmol/g. To ensure that the SO_2 was within the range of physiological concentration, SO_2 derivatives of 85 $mg.kg^{-1}.d^{-1}$ was used and we have demonstrated that such a concentration used in normal rats could not cause myocardial injury (Sun et al., 2010; Liang et al., 2011) and electrolyte imbalance. Our study verified that such a concentration of SO_2 derivatives could improve the decreased sulfite level in heart of rats and restore calcium homeostasis disturbance in rats with isoproterenol-induced myocardial injury.

From the above results, we can conclude that SO_2 at physiological concentration could improve myocardial injury induced by ISO in rats, and the mechanism might be related to the restoration of calcium overload by regulating the expression of SERCA and p-PLN/PLN in myocardial tissues. However, further studies are needed to explore its specific mechanism.

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