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# Dimethyl Sulfoxide Attenuates Hydrogen Peroxide-Induced Injury in Cardiomyocytes via Heme Oxygenase-1

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## ABSTRACT

The antioxidant property of dimethyl sulfoxide (DMSO) was formerly attributed to its direct effects. Our former study showed that DMSO is able to induce heme oxygenase-1 (HO-1) expression in endothelial cells, which is a potent antioxidant enzyme. In this study, we hypothesized that the antioxidant effects of DMSO in cardiomyocytes are mediated or partially mediated by increased HO-1 expression. Therefore, we investigated whether DMSO exerts protective effects against $H_2O_2$-induced oxidative damage in cardiomyocytes, and whether HO-1 is involved in DMSO-imparted protective effects, and we also explore the underlying mechanism of DMSO-induced HO-1 expression. Our study demonstrated that DMSO pretreatment showed a cytoprotective effect against $H_2O_2$-induced oxidative damage (impaired cell viability, increased apopototic cells rate and caspase-3 level, and increased release of LDH and CK) and this process is partially mediated by HO-1 upregulation. Furthermore, our data showed that the activation of p38 MAPK and Nrf2 translocation are involved in the HO-1 upregulation induced by DMSO. This study reports for the first time that the cytoprotective effect of DMSO in cardiomyocytes is partially mediated by HO-1, which may further explain the mechanisms by which DMSO exerts cardioprotection on $H_2O_2$ injury. J. Cell. Biochem. 115: 1159–1165, 2014. 

**KEY WORDS:** DIMETHYL SULFOXIDE; CARDIOMYOCYTES; HYDROGEN PEROXIDE; HEME OXYGENASE-1

Dimethyl sulfoxide (DMSO), a naturally derived pharmaceutical agent, has a variety of biological actions, which made it the target of pharmacological studies and the drug therapy vehicle for various diseases [Jacob and de la Torre, 2009]. Ischemia-reperfusion injury is an important clinical event in many disorders, with the resumption of blood flow, large amounts of reactive oxygen species (ROS) are produced. The increased level of ROS reacts with lipids in the cell and mitochondrial membranes, changing their permeability and integrity, and producing reversible or irreversible cell injury [Reilly et al., 1991]. One method of effective pharmacological therapy against ischemia-reperfusion injury is giving antioxidant agents, such as DMSO [Bulger and Maier, 2001]. The protective effects of DMSO against oxidative damage in ischemia-reperfusion injury have been demonstrated in the model of rat brain, ovary, testis, and lower limb [Koksal et al., 2006; Nagel et al., 2007; Ergun et al., 2010; Guimaraes et al., 2010]. The antioxidant property of DMSO was formerly attributed to its direct effects, since it is an efficient hydrogen-bound disrupter [Santos et al., 2003] and its specific reactivity with hydroxyl (OH) radicals [Kashino et al., 2010]. Interestedly, the results from our former study indicate that in human umbilical vein endothelial cells (HUVECs), DMSO is able to induce heme oxygenase-1 (HO-1) expression [Liang et al.,2012], which is a potent antioxidant and cytoprotective enzyme via its heme-derived metabolites [Li et al., 2007; Ryter and Choi, 2007]. Therefore, it seems that the protective effects of DMSO against oxidative stress may be mediated or partially mediated by increased HO-1 expression.

The increased levels of ROS are also involved in the process of myocardial ischemia-reperfusion injury [Droge, 2002]. In the ischemia-reperfusion model of rat heart, intraperitoneal and intracoronary injection of DMSO could reduce myocardium infarction size and the severity of postischemic left-ventricular dysfunction [Dmitriev et al., 2012]. On the other hand, the cardiac protective effects of HO-1 in vivo and vitro have been reported in many studies [Chen et al., 2011, 2013; Hull et al., 2013]. However, it remains unclear whether DMSO is able to upregulate HO-1 expression in cardiomyocytes, and whether DMSO-induced HO-1 is involved in its protective effects; therefore, the current studies were undertaken to clarify these questions.

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Many studies reported that mitogen-activated protein kinase (MAPK), nuclear factor-E2- related factor 2 (Nrf2), and phosphatidyinositol-3-kinase/Akt pathways are involved in signaling pathways of HO-1 expression [Chen et al., 2006]. These molecules involved in HO-1 upregulation are activated in an inducer or cell-specific manner [Masuya et al., 1998; Chen et al., 2006]. Our previous studies demonstrated that DMSO-induced HO-1 expression is related to the activation of JNKs and Nrf2 signaling pathways in HUVECs [Liang et al., 2012]. Along this line, we speculated that in cardiomyocytes, MAPKs and Nrf2 signal transduction may also be involved in the DMSO-induced HO-1 expression.

In the present study, we used cardiomyocytes injured by $H_2O_2$ as an oxidative stress model in vitro, to investigate the cytoprotective effects of DMSO, to examine whether HO-1 is involved in this process, and to explore the role of MAPKs and Nrf2 signal transduction in DMSO-induced HO-1 expression.

## MATERIALS AND METHODS

### CELL CULTURE

H9c2 cardiomyocytes (rat embryonic cardiomyoblast-derived) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured on dishes in medium with Dulbecco's modified Eagle's medium (DMEM) supplemented with antibiotics (100 mg/ml penicillin and 100 mg/ml streptomycin) at 37 °C in humidified air (5% $CO_2$). Then the cells were treated with different concentrations of $H_2O_2$ (50, 100, and 200 μM) and/or DMSO (0.1%, 0.2%, 0.4%, and 0.8%) to investigate their effects on cell viability and apoptosis. After that, before $H_2O_2$-induced injury, the cells were exposed to DMSO for 6 h with or without coincubation with ZnPP-IX (10 μM) for 1 h, PD98059 (25 μM) for 30 min, SP600125 (10 μM) for 30 min, or SB203580 (30 μM) for 30 min.

### WESTERN BLOT ANALYSIS

Cardiomyocytes were lysed in 1× sodium dodecyl sulfate cell lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 100 mg/ml phenylmethylsulfonyl fluoride, 1% SDS). Nuclear extracts were prepared by using the nuclear extract kit (Active Motif, CA) according to the manufacturer's instructions. Protein concentration was measured by BCA kit (Bio-color, Shanghai, China). Equal amounts of protein were separated and transferred to NC membranes (CNI, Canada). Subsequently, at room temperature, the membranes were blocked in Tris-buffered saline–Tween 20 containing 5% (w/v) nonfat dried milk for 1 h. After that, the membranes were incubated overnight with the respective concentration of primary antibody at 4 °C. Then, the filter was washed and probed with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. After washing, the blots were detected using enhanced chemiluminescence and the protein bands were quantitated with a densitometer.

### CELL VIABILITY ASSAY AND LDH AND CK MEASUREMENT

The methylthiozoyl tetrazolium (MTT) assay was used to determine cardiomyocytes viability. Briefly, after treatments, cells were washed and replaced with culture medium containing 0.5 μl/ml MTT. After 4 h incubation, medium was removed and isopropanol was added to solubilize formazan produced from MTT by viable cardiomyocytes. An automated microplate spectrophotometer was used to measure the absorbance at 570 nm. The LDH and CK levels in cellular supernatants were measured by ELISA kits following the manufacturer's instructions.

### ANALYSIS OF CASPASE-3 ACTIVATION

Fluorescein active caspase-3 staining kit (BioVision) was used to determine the activation of caspase-3. In brief, after different treatments, cardiomyocytes were collected and incubated on ice with 50 μl chilled lysate buffer for 10 min. Subsequently, 5 μl of caspase-3 substrate (DEVD- AFC, 1 μl) and 50 μl of 2× reaction buffer (containing 10 mM dithiothreitol) were added to each sample. The samples were then incubated at 37 °C for 2 h. The fluorescence at an excitation wavelength of 380 nm and an emission wavelength of 440 nm (SpectraFluor, TECAN, Sunrise, Austria) was measured to determine the activation of caspase-3.

### TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE-MEDIATED DUTP NICK END LABELING (TUNEL) STAINING

Terminaldeoxynucleotidyl-transferase (TdT-)mediated desoxyuridine-triphosphate (dUTP) nick end labeling (TUNEL) was used to detect cell apoptosis. Briefly, after different treatments, cardiomyocytes were fixed and permeated, and then incubated in the TUNEL reaction mixture. Morphological analysis was performed by a fluorescence microscopy (DM4000B, Leica, Wetzlar, Germany). In each sample, four random fields were selected and at least 100 cells were counted to calculate the apoptosis rate.

### HO-1 ENZYME ACTIVITY ASSAY

HO-1 activity was determined in microsomal fractions from cardiomyocytes as described previously [Ryter et al., 2000], by using HO-1 activity kit according to the manufacturer's instructions. HO-1 activity values were expressed as a nanomole of bilirubin formed per milligram of protein per hour.

### ELECTROPHORETIC MOBILITY SHIFT ASSAY (EMSA) TO DETECT NRF2-ANTI-OXIDANT RESPONSE ELEMENT BINDING

According to the manufacturer's instructions, nuclear extracts were prepared by the nuclear extraction kit (Active Motif, CA). The DNA–protein complexes were resolved by 5% non-denaturing PAGE. DNA binding activity for Nrf2 was determined by detecting the changes in intensity for the labeled oligonucleotides (50-TGG GGA ACC TGTGCT GAG TCA CTG GAG-30) binding to Nrf2.

### Nrf2 siRNA TRANSFECTION

In brief, cardiomyocytes were transfected with Nrf2 siRNA (Santa Cruz, CA) and mixed with Nrf2 siRNA transfection reagent (Santa Cruz) according to the manufacturer's instructions. After incubation at 37°C in 5% $CO_2$ for 30 h, cells were undergoing respective treatments. Subsequently, the samples were prepared for western blot analysis.

### STATISTICAL ANALYSIS

Data were reported as mean ± standard deviation (SD) of the results for at least three experiments. Experimental data were analyzed by

using one-way analysis of variance and Student's *t*-test. *P* values $<0.05$ were considered significant.

## RESULTS

### EFFECTS OF DMSO ON CELL VIABILITY AND HO-1 EXPRESSION AND ACTIVITY IN CARDIOMYOCYTES

Cardiomyocytes were incubated with different concentrations of DMSO-containing cell medium for 6 h. Cell viability was measured with the MTT assay. HO-1 protein expression was measured with Western blot analysis. The non-treated and DMSO-treated groups showed no significant difference in cell viability (Fig. 1A). We further investigated the effects of DMSO on HO-1 expression and activity in cardiomyocytes, and the results indicate that DMSO dose-dependently increased HO-1 expression and activity (Fig. 1B,C).

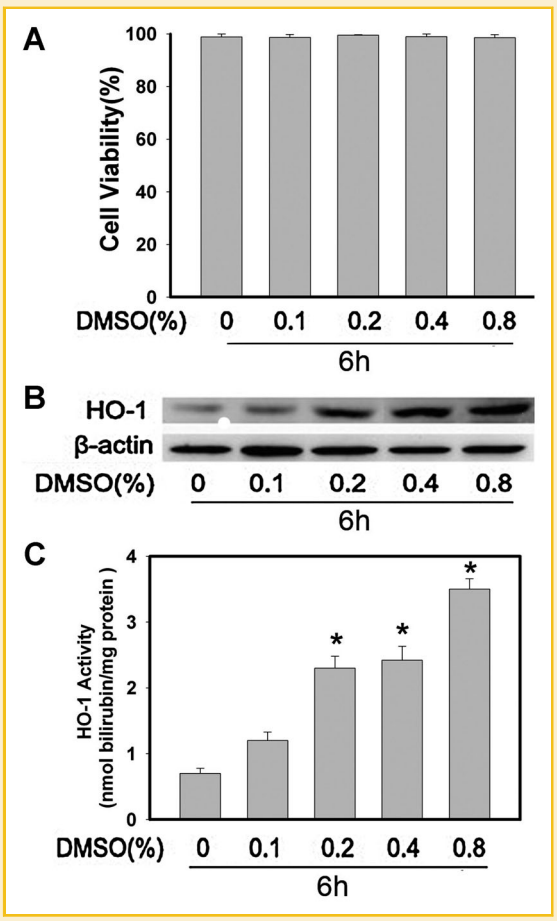


Fig. 1. The effects of DMSO on cell viability and heme oxygenase-1(HO-1) expression and activity in cardiomyocytes. Cells were exposed to various concentrations of DMSO for 6 h. Cell viability (A) was measured with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and HO-1 protein expression and activity were measured with western blot analysis and HO-1 activity kit, respectively (B,C). The non-treated and DMSO-treated groups showed no significant difference in cell viability, and HO-1 expression and activity were dose-dependently increased by DMSO. *$P < 0.05$ versus non-treated cells.

### EFFECTS OF DMSO ON CELL VIABILITY AND HO-1 EXPRESSION IN CARDIOMYOCYTES UNDER $H_2O_2$ INDUCED OXIDATIVE STRESS

Cardiomyocytes were treated with different concentrations of $H_2O_2$ (50, 100, and 200 μM) for 6 h, pretreated with or without 0.8% DMSO for 6 h. Cell viability was measured with the MTT assay, and HO-1 protein expression was measured with Western blot analysis. $H_2O_2$ significantly decreased cell viability at or above the concentration of 100 μM, which was reversed by DMSO pretreatment (Fig. 2A). HO-1 expression in the DMSO-pretreatment group was higher than that of control group and $H_2O_2$ alone group (Fig. 2B).

### PROTECTIVE EFFECTS OF DMSO ON CARDIOMYOCYTES AGAINST $H_2O_2$-INDUCED OXIDATIVE STRESS VIA HO-1

Cells were pretreated with 0.8% DMSO for 6 h, or pretreated with 10 μM ZnPP-IX (as an HO-1 inhibitor) for 1 h and 0.8% DMSO for

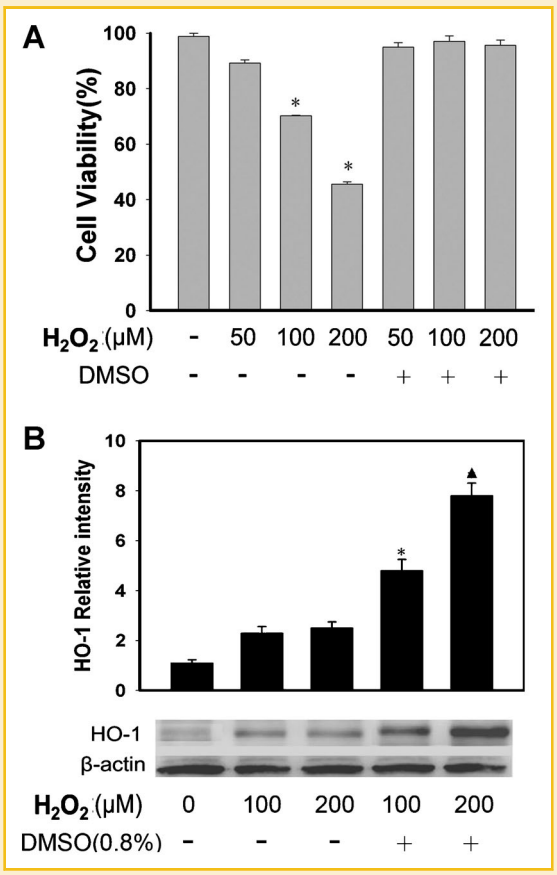


Fig. 2. The effects of DMSO on cell viability and HO-1- under $H_2O_2$-induced oxidative stress. Cardiomyocytes were treated with different concentrations of $H_2O_2$ (50, 100, and 200 μM) for 6 h, pretreated with or without 0.8% DMSO for 6 h. Cell viability was measured with the MTT assay, and HO-1 protein expression was measured with western blot analysis. DMSO pretreatment showed a cytoprotective effect against $H_2O_2$ oxidative stress in cardiomyocytes. $H_2O_2$ significantly decreased cell viability at or above the concentration of 100 μM. Cell viability of DMSO-pretreatment group was higher than that of $H_2O_2$ alone group (A) *$P < 0.05$ versus non-treated cells. HO-1 expression of DMSO-pretreatment group was higher than that of control group and $H_2O_2$ alone group (B) *$P < 0.05$ versus $H_2O_2$ 100 μM group, $P < 0.05$ versus $H_2O_2$ 200 μM group.

6 h. After different pretreatments, all cells were treated with 200 μM $H_2O_2$ for 6 h. Cell viability was measured with the MTT assay, TUNEL staining, and fluorescence detection of caspase-3 were used to investigate cell apoptosis, and HO-1 protein expression was measured with Western blot analysis. Pretreatment with DMSO significantly reversed the impaired viability, increased apopototic cell rate and activation of caspase-3 induced by $H_2O_2$. Treatment with ZnPP-IX abolished the DMSO-imparted protection on $H_2O_2$-induced injury (Fig. 3A–C). Furthermore, as shown in Figure 3D, the levels of LDH and CK were increased in the cells exposed to $H_2O_2$ injury as compared to the cells without treatment. However, pretreatment with DMSO reduced the $H_2O_2$ injury-enhanced levels of LDH and CK. Additionally, inhibition of HO-1 with ZnPP-IX also abolished the cytoprotective property of DMSO on release of LDH

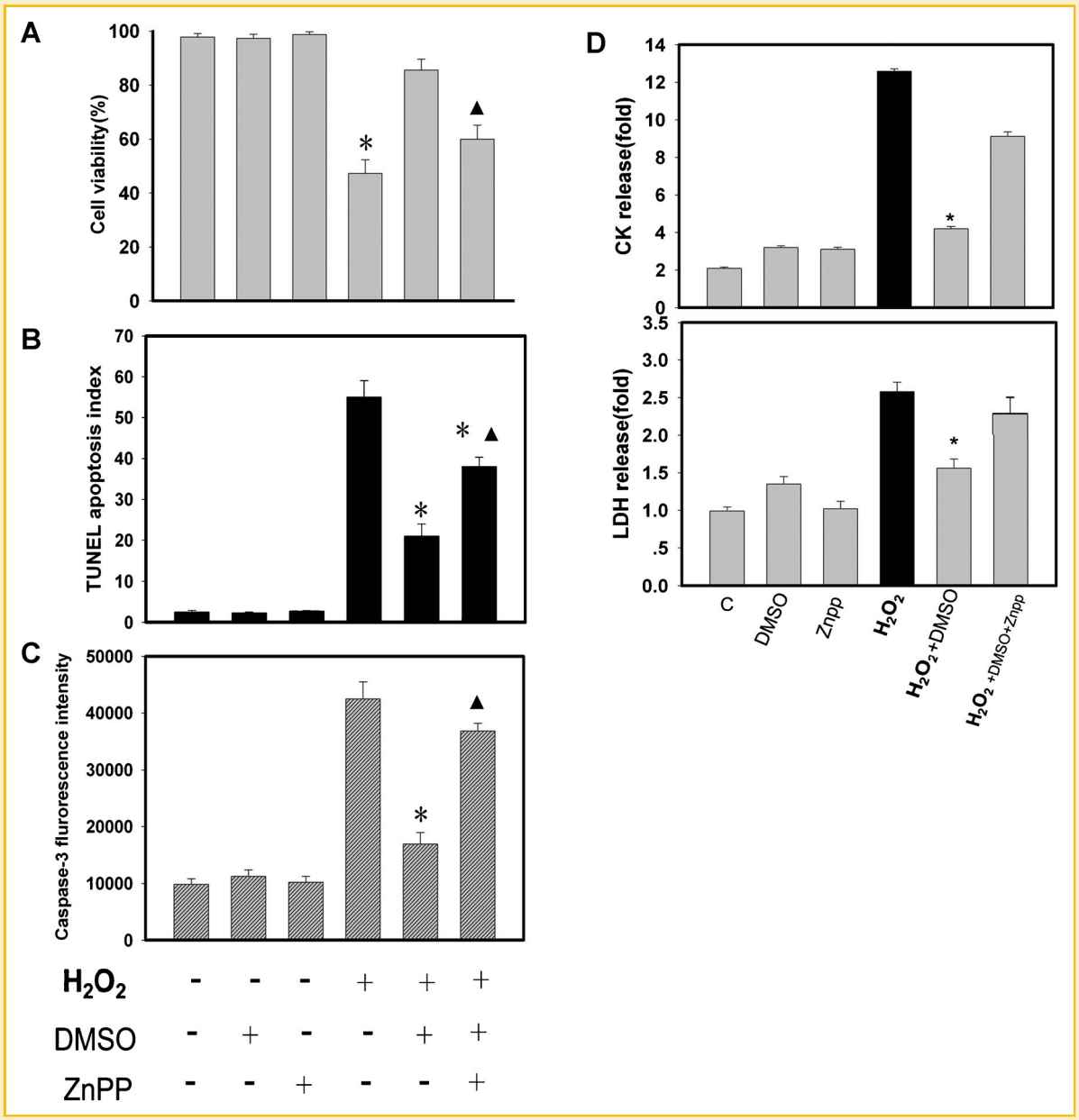


**Fig. 3.** Protective effects of DMSO on cardiomyocytes against $H_2O_2$-induced oxidative stress via HO-1. Cells were pretreated with 0.8% DMSO for 6 h, or pretreated with 10 μM ZnPP-IX (as an HO-1 inhibitor) for 1 h and 0.8% DMSO for 6 h. After different pretreatments, all cells were treated with 200 μM $H_2O_2$ for 6 h. Cell viability was measured with the MTT assay, TUNEL staining, and fluorescence detection of caspase-3 were used to investigate cell apoptosis, and HO-1 protein expression was measured with Western blot analysis. Pretreatment with DMSO significantly reversed the impaired viability, apopototic cells and activation of caspase-3, induced by $H_2O_2$. Treatment with ZnPP-IX partially abolished DMSO-imparted protection on $H_2O_2$-induced injury (A–C) *$P < 0.05$ versus $H_2O_2$ group, $P < 0.05$ versus $H_2O_2$ plus DMSO group. As shown in D, the levels of LDH and CK were increased in the cells exposed to $H_2O_2$ injury as compared to the cells without treatment. However, DMSO reduced the $H_2O_2$-enhanced levels of LDH and CK as compared to the cells treated by $H_2O_2$ alone. Additionally, inhibition of HO-1 with ZnPP-IX partially abolished the cytoprotective property of DMSO on release of LDH and CK. *$P < 0.05$ versus $H_2O_2$ group, $P < 0.05$ versus $H_2O_2$ plus DMSO group.

and CK (Fig. 3D). These data indicate that the protective effect of DMSO on cardiomyocytes against $H_2O_2$-induced oxidative stress is partially mediated by HO-1 upregulation.

### ROLE OF p38 MAPK, JNK, AND ERK IN HO-1 EXPRESSION INDUCED BY DMSO

The cells were exposed to DMSO for 6 h with or without coincubation with PD98059 (25 μM) for 30 min, SP600125 (10 μM) for 30 min, or SB203580 (30 μM) for 30 min, subsequently, all cells were treated with 200 μM $H_2O_2$ for 6 h, as shown in Figure 4A. The p38 MAPK pathway inhibitor SB203580 significantly reduced DMSO or DMSO plus $H_2O_2$-induced HO-1 expressions, whereas the JNK inhibitor SP600125 and ERK inhibitor PD98059 did not. Furthermore, DMSO alone or plus $H_2O_2$ increased P-p38 MAPK level, while P-ERK1/2 and P-JNK were less influenced by DMSO alone or plus $H_2O_2$ (Fig. 4B). These data indicate that under normal or $H_2O_2$-induced oxidative stress, DMSO-induced HO-1 expression is mediated through the activation of p38 MAPK pathways.

### ROLE OF Nrf2 TRANSLOCATION IN HO-1 EXPRESSION INDUCED BY DMSO

Cardiomyocytes were untreated or transfected with Nrf2-specific siRNA prior to 0.8% DMSO treatment. As illustrated in Figure 5A, DMSO and DMSO plus $H_2O_2$ significantly induced the Nrf2 translocation from cytoplasm to nuclei, while $H_2O_2$ alone did not. We further measured the DNA binding of Nrf2 by EMSA. As shown in Figure 5A, the DNA binding activity of Nrf2 was enhanced after treatment with DMSO and DMSO plus $H_2O_2$. Pretreatment of the cells with SB203580 inhibited the formation of Nrf2 banding complex induced by DMSO and DMSO plus $H_2O_2$. Finally, as shown in Figure 5B, enhanced protein expressions of HO-1 induced by DMSO or DMSO plus $H_2O_2$ were inhibited by Nrf2–siRNA. These results indicated that DMSO-induced HO-1 expression is mediated through Nrf2 translocation, and p38 MAPK may as a upstream signal of Nrf2 translocation.

## DISCUSSION

Cardiac H9c2 cells are a permanent cell line derived from embryonic rat hearts. It has been demonstrated that H9c2 cells show morphological characteristics similar to those of immature embryonic cardiocytes and preserve many elements of the electrical and hormonal signal pathway found in mature cardiac cells [Hescheler et al., 1991]. Therefore, this cell line has been widely used as a model for cardiocytes under many pathophysiologic conditions.

The results from present study indicated that DMSO have no effects on cardiomyocytes viability at the concentration of 0.1–0.8%. In human aortic and corneal endothelial cells, DMSO treatment also did not exert any toxic effect on cells even at the concentrations well above those used in our study [Giovanni et al., 1994; Camici et al., 2006]. Moreover, the DMSO concentrations used in our study are also below those used to induce cellular differentiation (1.25%) [Heidari et al., 2004].

We selected cardiomyocytes as our target cells, since myocardial ischemia/reperfusion (I/R) injury is a major challenge during many clinical events [Yellon and Hausenloy, 2007; Eltzschig and Eckle, 2011]. When the blood flow in ischemic tissues was restored, excessive pro-inflammatory cytokines and toxic compounds were produced. As the major toxic compounds generated at reperfusion, ROS activate multiple molecular cascades of inflammation [Yellon and Hausenloy, 2007; Eltzschig and Eckle, 2011]. HO-1 imparts cardioprotection mainly through the byproducts of HO-1 enzymatic

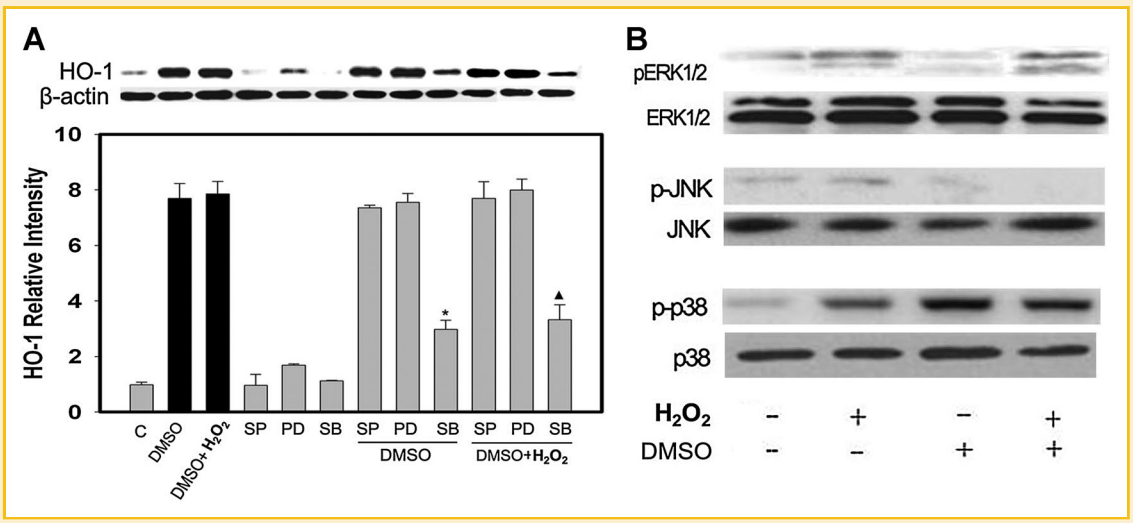


**Fig. 4.** Involvement of p38 MAPK in the expressions of HO-1 induced by DMSO. Cells were pretreated with 0.8% DMSO for 6 h or pretreated with PD98059 (25 μM) or SP600125 (10 μM) or SB203580 (30 μM) for 30 min. Then all cells were treated with 200 μM $H_2O_2$ for 6 h. HO-1 protein expression, total and P-p38 MAPK, total and P-JNK, total and P-ERK1/2 expressions were analyzed by Western blot, respectively. Results are representative of three independent experiments. p38 MAPK pathway inhibitor SB203580 significantly reduced DMSO-induced HO-1 expressions under normal or $H_2O_2$-induced oxidative stress, whereas the JNK inhibitor SP600125 and ERK inhibitor PD98059 did not (A). Furthermore, P-p38 MAPK level was increased by DMSO alone or DMSO plus $H_2O_2$ (B). *$P < 0.05$ versus $H_2O_2$ plus DMSO group.

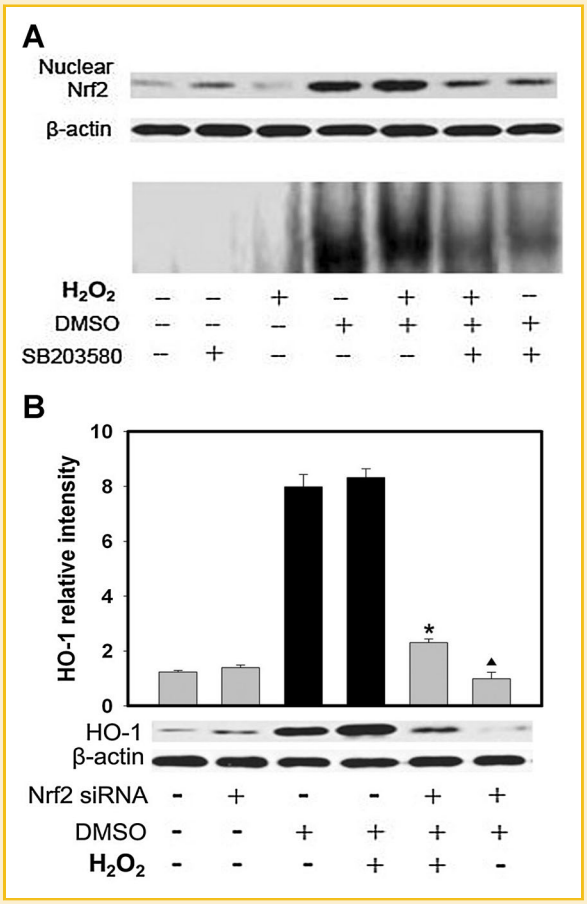


**Fig. 5.** DMSO-induced HO-1 expression is dependent on Nrf2 translocation. Cardiomyocytes with or without $H_2O_2$ exposure (6 h) were pretreated by 0.8% DMSO for 6 h, or SB203580 (30 $\mu$M) for 30 min. The nuclear lysates were prepared. Nrf2 proteins in nuclear extraction of cardiomyocytes were analyzed by using Western blotting. The DNA binding of Nrf2 was measured by EMSA. The blots are representative of three independent experiments. DMSO and DMSO plus $H_2O_2$ induced the Nrf2 translocation from cytoplasm to nuclei, while $H_2O_2$ alone did not. The DNA binding activity of Nrf2 was enhanced after treatment with DMSO and DMSO plus $H_2O_2$. Pretreatment of the cells with SB203580 inhibited the formation of Nrf2 banding complex induced by DMSO (A). Furthermore, cardiomyocytes were transfected with Nrf2-specific siRNA and then exposed to $H_2O_2$ (6 h) with or without pretreatment with 0.8%DMSO for 6 h. HO-1 protein expression was analyzed by Western blot. Densitometric analysis was performed after normalization with β-actin. *$P < 0.05$ versus DMSO group, $P < 0.05$ versus $H_2O_2$ plus DMSO group. All values are expressed as mean ± SD obtained from three independent experiments.

reaction, bilirubin and carbon monoxide [Vulapalli et al., 2002]. It has been demonstrated that HO-1 overexpression could prevent the I/R-induced cardiac dysfunction and apoptosis and regulate the apoptotic pathway genes like Bcl-2, Bax, and caspases [Vulapalli et al., 2002]. Therefore, pharmacological upregulation of HO-1 expression seems to have therapeutic potential in myocardial I/R injury [Lee et al., 2006; Hwa et al., 2012].

Our former study demonstrated that that DMSO is able to induce HO-1 expression in HUVECs, which identified DMSO as a HO-1 inducer. While we did not investigate whether HO-1 upregulation induced by DMSO could impart the protective effects on the cells under oxidative stress injury. For the purpose of investigating the protective effects of DMSO and involvement of HO-1 in cardiomyocytes, we performed the present study. We demonstrated for the first time that DMSO alone or DMSO plus $H_2O_2$ stimulated HO-1 protein expressions and activity in cardiomyocytes. Furthermore, DMSO pretreatment reversed the impaired cell viability, increased apopototic cells rate and caspase-3 level, and increased release of LDH and CK release induced by $H_2O_2$. These protective effects were partially abolished by ZnPP-IX, a HO-1 activity inhibitor. These data suggest that DMSO-induced cardioprotection is mediated by HO-1 upregulation in cardiomyocytes exposed to $H_2O_2$-induced oxidative injury.

Many signaling pathways were involved in HO-1 transcriptional regulation, which are depending on the cell type- and inducer-specific features [Chen et al., 2006; Wu et al., 2006; Cooper et al., 2007; Liu et al., 2007]. Similarly, previous studies have shown that HO-1 expression induced in cardiomyocytes exposed to oxidative stress or hypoxia is mediated by the activation of p38 MAPK [Kacimi et al., 2000; Schulz et al., 2002]. On the other hand, in cardiac ischemic models, numerous studies also demonstrated a crucial role of p38 MAPK [Bassi et al., 2008]. Based on these findings, results from the present studies show that the inhibition of p38 MAPK by SB203580, but not inhibition of ERK or JNK, significantly eliminated the upregulation of HO-1 expression induced by DMSO alone or DMSO plus $H_2O_2$. Furthermore, DMSO alone or plus $H_2O_2$ significantly increased the levels of P-p38 MAPK but not the levels of p-ERK or p-JNK. In addition, $H_2O_2$ (200 μmol/L) alone or DMSO plus $H_2O_2$ stimulates ERKs phophorylation slightly, which is consistent with our results in the study of HUVECs [Liang et al., 2012]. These data indicated that signaling mechanisms involved in p38 MAPK phosphorylation is responsible for HO-1 gene activation induced by DMSO under normal or oxidative stress conditions.

Nrf2 served as an important role in ARE-mediated antioxidant gene expression and the transcriptional activation of HO-1 gene [Mann et al., 2007]. However, the role of Nrf2/ARE modulated by DMSO in its cardioprotection remains unknown. The present studies clarified that the translocation of Nrf2 and activation of Nrf2/ARE are essential for DMSO-induced HO-1 upregulation in cardiomyocytes. First, DMSO and DMSO plus $H_2O_2$ induced Nrf2 translocation. And in the EMSA that was conducted using a binding sequence for Nrf2, no binding complex was detected in the untreated cardiomyocytes, but the nuclear translocation and DNA binding of Nrf2 were increased by DMSO and DMSO plus $H_2O_2$. Furthermore, the expressions of HO-1 protein induced by DMSO and DMSO plus $H_2O_2$ were inhibited by Nrf2-siRNA transfection. These data confirm that DMSO significantly induced Nrf2 translocation from cytoplasm into nuclei and inducing the upregulation of HO-1. Besides, the nuclear Nrf2 levels and enhanced binding complex were dependent on activation of p38 MAPK, since p38 MAPK inhibitor SB203580 inhibited the formation of binding complex induced by DMSO and DMSO plus $H_2O_2$ injury, and these results may also indicate p38 MAPK is an upstream signal of Nrf2 translocation.

In summary, our present experiments indicate that DMSO upregulates the expressions of HO-1 expression and activity, which protects cardiomyocytes against $H_2O_2$ injury via activation of p38 MAPK pathway and nuclear translocation of Nrf2. Our results further explained the mechanisms by which DMSO exerts

cardioprotection on $H_2O_2$ injury, which may represent useful tools for the development of a new drug for treatment of heart diseases related to oxidative stress injury, such as ischemic heart diseases.

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