Anti-oxidation of Tanshinone ⅡA and Prohibitin on Cardiomyocytes

YANG Ping, JIA Yu-hua*, LI Jie, ZHOU Feng-hua, LI Li-jun

Southern Medical University, Guangzhou 510515, China

Abstract: **Objective** To investigate the anti-apoptotic mechanism of tanshinone ⅡA and the function of prohibitin (PHB) on myocardial cells apoptosis induced by hydrogen peroxide (H_2O_2). **Methods** Myocardial cells were primary cultured neonate rat were cultured in medium with 200 μmol/L H_2O_2, and the medium was supplemented with tanshinone ⅡA (1×10^{-4} mol/L) in advance for 24 h. PHB in myocardial cells was knocked down by RNA interference, and the expression level of PHB was determined by Western blotting analysis. Flow cytometric analysis was used to detect apoptosis rate, intracellular calcium concentration ($[Ca^{2+}]_i$), and mitochondrial membrane potential (MMP). **Results** H_2O_2-mediated cell apoptosis resulted in activation of PHB, increasing of $[Ca^{2+}]_i$, and decreasing of MMP. Tanshinone ⅡA profoundly inhibited myocardial cell apoptosis induced by H_2O_2, decreased $[Ca^{2+}]_i$, and increased MMP. Specific silence of PHB by siRNA down-regulated the expression level of PHB, increased apoptosis rate and $[Ca^{2+}]_i$, and decreased MMP. **Conclusion** The results demonstrate that tanshinone ⅡA could attenuate apoptosis induced by H_2O_2, and the activation of PHB induced by H_2O_2 is the major regulatory pathway of cyto-protective gene expression against oxidative stress.

Key words: calcium overload; myocardial cell; oxidative stress; prohibitin; tanshinone ⅡA
DOI: 10.3969/j.issn.1674-6384.2010.03.007

Introduction

Ischemia-reperfusion (IR) injury secondary to revascularization with thrombolytic drugs or interventional procedures, such as percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass grafting (CABG) is an emerging problem (Schäfer *et al*, 2002). Although timely reperfusion of acute ischemic myocardium is essential for myocardial salvage, reperfusion may also result in a unique form of myocardial damage. A number of mechanisms have been proposed to mediate reperfusion injury, of which perhaps the most important is the formation of oxygen radicals. A large number of studies have shown that free radical scavenger could prevent or scavenge damaging oxidants and free radicals (Sun, 2007).

Tanshinone ⅡA is one of the major lipid-soluble constituents of *Salvia miltiorrhiza* Bunge known as *Danshen* (Wang *et al*, 2008), which has long been used for prevention and treatment of cardiovascular diseases in China (Chen, Lu, and Zheng, 2009). Accumulating studies show that tanshinone ⅡA possesses many biological and pharmacologic properties (Kimm *et al*, 2004; Du *et al*, 2005) mainly depending on its anti-oxidative effects (Tang *et al*, 2007). In our previous study we have shown in a rat model that tanshinone ⅡA could attenuate arrhythmia induced by myocardial IR by inhibition of nitric oxide (NO) and platelet adhesion molecules, such as CD41 and CD62P (Sun, Jia, and Chen, 2000; Jia, Sun, and Chen, 2002). The purpose of this article is to investigate the effect of tanshinone ⅡA on apoptosis induced by hydrogen peroxide (H_2O_2) in primary cultured rat cardiomyocytes *in vitro* and underlying molecular mechanisms.

Prohibitin (PHB), a highly conserved protein, is localized in the inner mitochondrial membrane. The function of PHB is associated with stabilization of mitochondrial function, suppression of tumor cell proliferation, regulation of apoptosis and cell proliferation, protection against aging, and so on (Mishra *et al*, 2005). Recently, we used proteomic analysis to detect differentially

* Corresponding author: Jia YH, male, mainly occupied with the basic and clinical research of cardiovascular diseases with combination of Chinese traditional and Western medicine Address: School of Traditional Chinese Medicine, Southern Medicial University, Tonghe, Guangzhou
Email: jyh@fimmu.com Tel: +86-20-6164 8250
Received: October 14, 2009; Revised: December 26, 2009; Accepted: January 15, 2010
Support: National Natural Sciences Foundation of China (30572435)

expressed proteins in IR hearts, we finally identified that PHB was in high expression in the IR hearts compared with the control hearts, the result was consistent with the study by Kim *et al* (2006). However, the specific cellular function of PHB in myocardial cells has not been clearly elucidated. The goal of this study was to investigate the anti-apoptotic mechanism of tanshinone ⅡA, the regulation and the function of PHB during injury induced by H_2O_2. In order to elucidate the function of PHB, RNA interference (RNAi) was used to knock down the expression of PHB.

Materials and methods

Materials

SPF Wistar neonatal rats (1–3 d old) were purchased from Experimental Animal Center of Southern Medical University. Trypsin and DMEM culture media were purchased from Gibico (USA). siPORT NeoFX reverse transfection kit was purchased from Ambion (USA). Fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Biotech Co., Ltd. Tanshinone ⅡA was purchased from China Pharmaceutical Biological Products Analysis Institute. PHB antibody, goat anti-mouse IgG-HRP, and PHB siRNA, and transfection reagent were purchased from Santa Cruz (USA). ECL chemiluminescence kit and protein excretion kit, Fluo-3/AM, Rhodamine 123 (Rh123), and AnnexinV-FITC cell apoptosis detection kit were purchased from Nanjing KeyGen Biotech Co., Ltd.

Primary cardiomyocyte culture

Primary cardiac myocyte culture from neonatal Wistar rats were prepared according to the procedure described by Goldenberg *et al* (2003). In brief, the hearts were surgically removed from (1–3) d old Wistar rats, minced into (1–2) mm^3 pieces and then washed for three times with *D*-Hanks. The minced tissue was put into a 50 mL tube containing 0.1% trypsin solution for trypsinization, 37 ℃ for 6 min. Supernatant was dispersed at first time. Then, the tissue was subjected to seven to ten cycles' trypsinization, until all the tissues were trypsinizd. Supernatants from each cycle were collected and centrifuged twice with DMEM with 5% fetal bovine serum at 1000 r/min. For selective enrichment with cardiac myocytes, dissociated cells were preplated for 1 h, during which period the non-myocytes attached readily to the bottom of the culture dish. In the end, the cell pellet was resuspended in DMEM supplemented with 15% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin. Bromodeoxyuridine (0.1 mmol/L) was added during the first two days to prevent the proliferation of non-myocytes. The cells were cultured in DMEM containing 15% FBS, 150 mmol/L HEPES, 100 U/mL penicillin, and 100 g/mL streptomycin. Cultured cells were then incubated at 37 ℃ in humidified atmosphere of 95% air and 5% CO_2, medium was replaced every 24 h. More than 95% of cells were cardiomyocytes, as evidenced by immunostaining with α-sarcomeric actin antibody. For all experiments, myocardial cells were grown to 80%–90% confluence and made quiescent by starvation for 24 h. Tanshinone ⅡA was added 24 h before treatment with H_2O_2.

Evaluation of cytotoxicity

Cells were cultured in a 96-well microplate. Increasing concentration of H_2O_2 (50, 100, 200, 400, and 800 μmol/L) and tanshinone ⅡA (1×10^{-5}, 5×10^{-5}, and 1×10^{-4} mol/L) were added to the culture. After 2 h incubation, cytotoxicity of H_2O_2 was assessed by MTT assay. After 24 h incubation, cytotoxicity of tanshinone ⅡA was assessed by MTT assay. MTT solution (5 mg/mL) was added to each well in the assay, and plates were incubated at 37 ℃ for 4 h in the dark. After 4 h, the medium was removed, and 200 μL DMSO was added to each well and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at room temperature to ensure that all crystals were dissolved, the plates were read on ELISA plate reader at OD 570 nm.

RNA intervention

Adherent cells were trypsinized and diluted in normal growth medium to 1×10^5 cells per mL. Diluted (2–8) μL of siRNA transfection reagent A and B into 100 μL siRNA transfection medium. Mixed gently by pipetting the solution up and down and incubate the mixture (15–45) min at room temperature. Diluted siRNA appropriately with siRNA dilution buffer. Washed the cells once with 2 mL of siRNA transfection medium. For each transfection, 0.8 mL siRNA transfection medium was added to each tube containing the siRNA transfection reagent mixture (solution A + solution B). The mixture was mixed gently and overlay onto the washed cells. The cells were incubated (5–7) h at 37 ℃ in a CO_2 incubator. Normal growth medium (1 mL) containing two times the normal serum and antibiotics

concentration was added. The cells were incubated for an additional (18–24) h. The medium was aspirated and replaced with fresh 1 × normal growth medium. The effects of RNAi were assayed by Western blotting after the addition of fresh medium in the step above.

Apoptosis analysis

The cell apoptosis was analyzed by flow cytometry after staining DNA with propidium iodide (PI) and Aannexin V-FITC. At the indicated time points after 5 μg/mL of Tanshinone ⅡA treatment, cells were trypsinized, rinsed with ice-cold PBS, and fixed in 70% ethanol for overnight at −20 ℃. After the fixation, cells were washed with ice-cold PBS and treated with 200 μL of RNase A (1 mg/mL) at 37 ℃ for (30–60) min. Cells were then incubated with 800 μL of PI staining buffer (0.1 mg/mL PI and 1% Triton X-100 in PBS) at 4 ℃ in the dark for 30 min. Afterwards, cells were analyzed by FACS on a Calibur Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ).

Measurement of MMP

Myocardial cells were trypsinized and harvested, and diluted to a concentration of 1×10^5 cells per mL. After washing the cells twice in PBS, they were loaded with Rh123 (100 μg/L), and incubated at 37 ℃, 5% CO_2 for 45 min in darkness. MMP was analyzed by flow cytometry (λ_{em}= 488 nm, λ_{ex} = 425 nm), and fluorescence intensities were measured using Cell Quest software. MMP was expressed by mean fluorescence intensities of the positive cells.

Intracellular calcium measurement

Adherent cells were trypsinized and harvested, and diluted to a concentration of 1×10^5 cells per mL. After washing the cells twice in PBS, they were loaded with Fluo-3/AM at a terminal concentration of 10 μmol/L, and incubated at 37 ℃, 5% CO_2 for 30 min in darkness. Then cells were washed twice in PBS, Ca^{2+} responses were observed and evaluated by means of flow cytometry (FACSort, Becton Dickinson) within 60 min (λ_{em}= 527 nm, λ_{ex}= 506 nm). Mean channel fluorescence intensities were calculated using Cell Quest software. Intracellular calcium concentration ($[Ca^{2+}]_i$) was calculated by the formula:

$$[Ca^{2+}]_i = k_d [(F - F_{min}) / (F_{max} - F)]$$

k_d represents dissociation constant of Fluo-3, the value is 400 nmol·L^{-1}, F represents channel fluorescence intensities of each sample, F_{max} and F_{min} represents channel fluorescence intensities of each sample that was loaded with 0.1% Triton X-100 or 5 mmol·L^{-1} EGTA, respectively

Western blotting

Protein concentration was determined by BCA protein assay kit. Protein (50 μg) was subjected to 10% SDS–PAGE, and then transferred to nitrocellulose membrane. After 1 h blocking with non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBST), the membrane was incubated with 1∶800 primary antibody for PHB overnight at 4 ℃. After washing, the membrane was incubated with HRP-conjugated goat anti-mouse secondary antibody (1∶1000) for 1 h at room temperature. After washing, the blots were developed using an enhanced chemi-luminescence detection kit. The membrane was then reprobed with β-actin antibody. The blotting was quantified by densitometry and normalized by using β-actin signal in order to correct any error during sample preparation and protein loading. The intensity of bands obtained from Western blotting images were captured and estimated with Kodak software.

Statistical analysis

The data presented as the means ± SEM. Statistical analysis was made by one-way ANOVA followed by LSD test. A value of $P < 0.05$ was considered statistically significant.

Results

Effects of tanshinone ⅡA on H_2O_2-induced injury in myocardial cells

Myocardial cells were exposed to various concentration of H_2O_2 (50–800) μmol/L for 2 h, and the cell cytotoxicity was determined by MTT assay. As shown in Fig. 1A, H_2O_2 significantly inhibited the viability of myocardial cells in a dose-dependent manner. Additionally, cell viability was significantly improved in the presence of tanshinone ⅡA in a dose-dependent manner (Fig. 1B).

Effect of tanshinone ⅡA and siRNA on cell apoptosis related changes

To clarify whether the effect of tanshinone ⅡA (1×10^{-4} mol/L) was related to the alteration of myocardial cell apoptosis status, we examined cell apoptosis rate, $[Ca^{2+}]_i$, and MMP. As shown in Table 1, apoptosis rate and $[Ca^{2+}]_i$ were significantly increased, and MMP levels were significantly down-regulated by treatment with H_2O_2 (200 μmol/L) for 2 h; Pretreatment with tanshinone ⅡA (1×10^{-4} mol/L) for 24 h provided significant protection

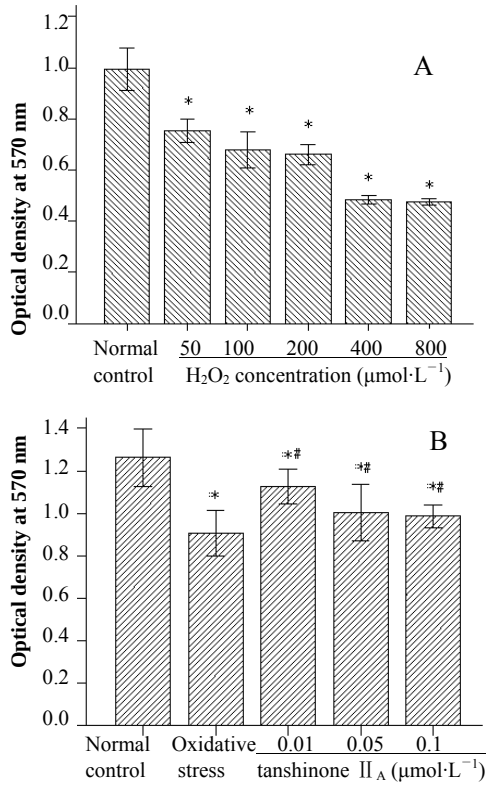

Fig. 1 Myocardial cells treated with H_2O_2 and tanshinone ⅡA at different concentration

A: Myocardial cells treated with 50, 100, 200, 400, and 800 μmol·L^{-1} H_2O_2 for 2 h and pretreated

B: Myocardial cells pretreated by 1×10^{-5}, 5×10^{-5}, and 1×10^{-4} mol·L^{-1} tanshinone ⅡA for 24 h before addition of H_2O_2

*$P < 0.05$ *vs* control group

#$P < 0.05$ *vs* the oxidative stress group

Both cell cytotoxicities by H_2O_2 and the protective effect by tanshinone ⅡA were assessed by the MTT assay, and the absorbance was read at 570 nm

against down-regulation of apoptosis rate, $[Ca^{2+}]_i$ caused by H_2O_2, while the MMP levels were significantly up-regulated. siRNA against PHB treatment significantly increased apoptosis rate and $[Ca^{2+}]_i$, and decreased MMP in myocardial cells, the values were more noticeable in siRNA+ oxidative stress group.

Effects of tanshinone ⅡA and siRNA on PHB protein expression in myocardial cells

In this study, we set the ratio of the gray value between PHB protein and β-actin as 1, then the ratio was 1.691 ± 0.041 in oxidative stress other groups, 1.261 ± 0.058 in tanshinone ⅡA group, 0.553 ± 0.020 in siRNA group, 0.576 ± 0.019 in siRNA + oxidative stress group, and 0.992 ± 0.033 in negative control siRNA. The expression of PHB increased remarkably under oxidative stress conditions (Fig. 2). However, H_2O_2-induced increase in the quantity of PHB was attenuated by tanshinone ⅡA pretreatment compared with oxidative stress group. siRNA against PHB treatment specifically decreased PHB protein, indicating that siRNA was effective in down-regulating the protein expression. The quantity of PHB was not affected by negative control siRNA.

Table 1 Effect of tanshinone ⅡA and siRNA against PHB on H_2O_2-induced changes in myocardial cells

Groups	Apoptosis rate/ %	$[Ca^{2+}]_i$/ (nmol·L^{-1})	MMP
Normal control	2.530±0.183	94.560±4.511	83.650±1.793
Oxidative stress	44.820±2.430*	277.273±7.818*	47.543±1.341*
tanshinone ⅡA	17.477±1.196*▲	133.350±8.635*▲	76.530±1.206*▲
siRNA	13.560±1.032*▲	133.593±10.903*▲	79.3200±0.805*▲
siRNA+Oxidative stress	58.273±1.225*▲	336.648±7.852*▲	40.9867±2.182*▲
negative control siRNA	2.657±0.125▲	92.217±5.249▲	83.7033±0.981▲

*$P < 0.05$ *vs* normal control group; ▲$P < 0.05$ *vs* oxidative stress group

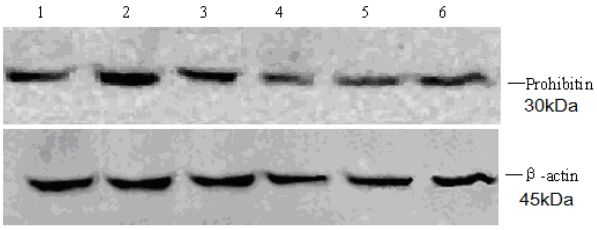

Fig.2 Effect of tanshinone ⅡA on H_2O_2-induced changes in PHB protein and siRNA against prohibitin

1: The quantity of PHB protein in normal control group

2: The quantity of PHB protein in oxidative stress group

3: The quantity of PHB protein in tanshinone ⅡA group

4: The quantity of PHB protein in siRNA group

5: The quantity of PHB protein in siRNA + oxidative stress group

6: The quantity of PHB protein in siRNA negative control group

Discussion

IR injury is a well-known phenomenon following CABG, thrombolytic therapy and cardiopulmonary bypass therapy (CPBT). A number of mechanisms have been proposed to mediate reperfusion injury, of which the most important is the formation of reactive oxygen species (Wang *et al*, 2008; Giacomo and Antonio, 2007; Fischer *et al*, 2006). Myocardial IR injury not only results in cell necrosis, but also induces cell apoptosis (Bognar *et al*, 2006) which can be triggered through different mechanisms in response to both intracellular and extracellular signals. The excessive apoptosis leads to the decreasing of cell number, damages of cell structure and cell function. There is strong evidence that

elevated $[Ca^{2+}]_i$ is the initial factor of apoptosis (Sodha *et al*, 2008) and inhibition of calcium concentration may prevent the process of apoptosis (McAinsh and Pittman, 2009). MMP decreasing occurs in the early period of apoptosis, which means that the mitochondrial membrane has been damaged. In this study, we found that exogenous H_2O_2 at a concentration of 200 μmol/L was associated with a remarkable increase of apoptosis rate and $[Ca^{2+}]_i$ and a significant decrease of MMP. In this study, we found that tanshinone ⅡA inhibited the alteration of $[Ca^{2+}]_i$ and MMP induced by H_2O_2, so that cell apoptosis rate was remarkably reduced. Therefore, tanshinone ⅡA was proved to have protective effect on myocardial cells against apoptosis induced by oxidative stress.

PHB was first found as a tumor suppressor gene, for its significant anti-proliferative activity (Galang, Sasaki, and Maulik, 2000), so it is also called anti-proliferative protein. PHB protein is evolutionally conserved, it is localized in the inner mitochondrial membrane (Dell'Orco *et al*, 1996). The nuclear localization of PHB has also been reported in a variety of cell lines (Czarnecka *et al*, 2006; Winter, Kämäräinen, and Hofmann, 2007). A variety of functions of PHB have been suggested, including a role in cell cycle regulation, differentiation, and controlling apoptosis. The natural substrates of PHB include cytochrome oxidase, mitochondrial complex Ⅰ, and so on. PHB proteins also play crucial roles in the mitochondria. Recently, human PHB1 was reported to associate with mitochondrial complex Ⅰ, suggesting a regulation of mitochondrial respiratory activity. PHB has been shown to function as chaperones that stabilize newly synthesized mitochondrial protein (Kasashima *et al*, 2006). PHB has also participated in the production of calsium-dependent ATP, suggesting that PHB plays a role in regulation of energy metabolism (Artal-Sanz *et al*, 2003). Currently, the finding of the relationship between PHB protein and diseases mostly studied by proteomics (Ferrer *et al*, 2007; Saridaki and Panayotou, 2005; Hsieh *et al*, 2006; Li *et al*, 2004; Mi *et al*, 2006). Our previous study confirmed that IR injury led to a increase of protein in rats, the result is in accordance with Kim's study (Kim *et al*, 2006). The present study is that PHB protein shows an anti-apoptosis effect against H_2O_2-induced injury.

RNAi is a process of sequence specific degradation of RNA in the cytoplasm of eukaryotic cells that is induced by double stranded RNA, and causes target RNA post-transcription gene silencing (Gonzalez-Gonzalez, Lopez-Casas, and Del, 2008) Currently, the RNAi technology is widely utilized in the functional genomic studies and intense research is being carried out around the world to exploit siRNA for therapeutic purposes. In the present study, we have evaluated the potential of specific PHB siRNA to inhibit PHB protein expression in myocardial cells. As a result, a remarkable inhibition of PHB was observed after siRNA against PHB treatment, although the inhibition was not totally, and was not as satisfactory as we thought. The reason may be that siRNA transfection efficiency varied from one cell type to another, it is not effective in primary cultured myocardial cells. Anyway, the inhibition of PHB by siRNA caused a significant increase in cell apoptosis rate and $[Ca^{2+}]_i$, and a decrease in MMP, the changes were more remarkable in siRNA + oxidative stress group. This result indicated that PHB proteins show protective effect against oxidative stress. A recent report demonstrated that PHB played an important role in combating oxidative stress in human and animal models of inflammatory bowel disease (Theiss *et al*, 2007). Our study shows the similar function of anti-oxidative activity of PHB.

Free radicals have been suggested as potentially important causative agents of aging and several human diseases. Therefore, one of the strategies is to look for natural products from plants for combating free radical-induced pathological status. Tanshinone ⅡA is one of the major lipophilic components of the herbal medicine *S. miltiorrhiza*. A variety of studies show that tanshinone ⅡA is a natural antioxidative drug against lipid peroxidation (Zhang *et al*, 2003; Zhang *et al*, 2004). In the present study, the outstanding anti-oxidative activity of tanshinone ⅡA was observed in myocardial cells. Besides, a decrease in the amount of PHB protein was also obsereved after pretreatment of tanshinone ⅡA. The result seems paradox, as PHB protein proved to be beneficial for myocardial cells against oxidative stress. Actually, that is not the case. The increase of PHB protein is a compensatory response to the oxidative stress (Kim *et al*, 2006). A current study showed that the acute phase cytokine interleukin-6 (IL-6) increased PHB protein and mRNA abundance and induced PHB promoter

activation, and IL-6 responsiveness are affected by signal transducer and activator of transcription 3 (STAT3). Many studies support that a lot of inflammatory factors participate in the process of IR (Podgoreanu *et al*, 2005), and tanshinone ⅡA could effectively down-regulate the amount of these inflammatory factors (Jang *et al*, 2003), such as IL-6, TNF-α, and so on. In this study, we found that the level of PHB protein level in myocardial cells was down-regulated by tanshinone ⅡA pretreatment under oxidative stress condition, while the amount of the protein was still larger than that in the normal control group. There may be two reasons for this case. First, tanshinone ⅡA probably down-regulated PHB by attenuating oxidation reaction that subsequently led to a decrease in the amount of PHB protein. Second, tanshinone ⅡA may down-regulate PHB directly. In this case, we hypothesize that tanshinone ⅡA down- regulates the expression of PHB by inhibiting the activation of inflammatory factors in myocardial cells under oxidative stress or acting directly on PHB. As for the real mechanism, we need further studies to confirm this suppose.

Reference

Artal-Sanz M, Tsang WY, Willems EM, Grivell LA, Lemire BD, van der Spek H, Nijtmans LG, 2003. The mitochondrial prohibitin complex is essential for embryonic viability and germline function in *Caenorhabditis elegans*. *J Biol Chem* 278(34): 32091-32099.

Bognar Z, Kalai T, Palfi A, Hanto K, Bognar B, Mark L, Szabo Z, Tapodi A, Radnai B, Sarszegi Z, Szanto A, Gallyas FJ, Hideg K, Sumegi B, Varbiro G, 2006. A novel SOD-mimetic permeability transition inhibitor agent protects ischemic heart by inhibiting both apoptotic and necrotic cell death. *Free Radic Biol Med* 41(5): 835-848.

Chen L, Lu Y, Zhang SZ, 2009. Integration effect on pharmacological component in *Salvia miltiorrhiza*. *Chin Tradit Herb Drugs* 40(3): 476-479.

Czarnecka AM, Campanella C, Zummo G, Cappello F, 2006. Mitochondrial chaperones in cancer: from molecular biology to clinical diagnostics. *Cancer Biol Ther* 5(7): 714-720.

Dell'Orco RT, McClung JK, Jupe ER, Liu XT, 1996. Prohibitin and the senescent phenotype. *Exp Gerontol* 31(1-2): 245-252.

Du JR, Li X, Zhang R, Qian ZM, 2005. Tanshinone inhibits intimal hyperplasia in the ligated carotid artery in mice. *J Ethnopharmacol* 98: 319-322.

Ferrer I, Perez E, Dalfó E, Barrachina M, 2007. Abnormal levels of prohibitin and ATP synthase in the substantia nigra and frontal cortex in Parkinson's disease. *Neurosci Lett* 415(3): 205-209.

Fischer UM, Antonyan A, Bloch W, Mehlhorn U, 2006. Impact of antioxidative treatment on nuclear factor kappa-B regulation during myocardial ischemia-reperfusion. *Interact Cardiovasc Thorac Surg* 5(5): 531-535.

Galang N, Sasaki H, Maulik N, 2000. Apoptotic cell death during ischemia/reperfusion and its attenuation by antioxidant therapy. *Toxicology* 148(2-3): 111-118.

Giacomo CG, Antonio M, 2007. Melatonin in cardiac ischemia/ reperfusion-induced mitochondrial adaptive changes. *Cardiovasc Hematol Disord Drug Targets* 7(3): 163-169.

Goldenberg I, Shainberg A, Jacobson KA, Shneyvays V, Grossman E, 2003. Adenosine protects against angiotensin II-induced apoptosis in rat cardiocyte cultures. *Mol Cell Biochem* 252(1): 133-139.

Gonzalez-Gonzalez E, Lopez-Casas PP, Del MJ, 2008. Gene silencing by RNAi in mouse Sertoli cells. *Reprod Biol Endocrinol* 6(1): 29.

Hsieh SY, Shih TC, Yeh CY, Lin CJ, Chou YY, Lee YS, 2006. Comparative proteomic studies on the pathogenesis of human ulcerative colitis. *Proteomics* 6(19): 5322-5331.

Jang SI, Jeong SI, Kim KJ, Kim HJ, Yu HH, Park R, Kim HM, You YO, 2003. Tanshinone ⅡA from *Salvia miltiorrhiza* inhibits inducible nitric oxide synthase expression and production of TNF-alpha, IL-1beta and IL-6 in activated RAW 264.7 cells. *Planta Med* 69(11): 1057-1059.

Jia YH, Sun XG, Chen YY, 2002. Ding Xin recipe and tanshinone ⅡA attenuates ischemia-reperfusion arrhythmia and the mechanism of platelet adhesion molecules. *J Tradit Chin Med* 43(2): 140-143.

Kasashima K, Ohta E, Kagawa Y, Endo H, 2006. Mitochondrial functions and estrogen receptor-dependent nuclear translocation of pleiotropic human prohibitin 2. *J Biol Chem* 281(47): 36401-36410.

Kim N, Lee Y, Kim H, Joo H, Youm JB, Park WS, Warda M, Cuong DV, Han J, 2006. Potential biomarkers for ischemic heart damage identified in mitochondrial proteins by comparative proteomics. *Proteomics* 6(4): 1237-1249.

Kimm HH, Kim JH, Kwak HB, Huang H, Han SH, Ha H, Lee SW, Woo ER, Lee ZH, 2004. Inhibition of osteoclast differentiation and bone resorption by tanshinone ⅡA isolated from *Salvia miltiorrhiza* Bunge. *Biochem Pharmacol* 67: 1647-1656.

Li R, Liu XL, Du QF, Tian H, Song LL, Zhang Y, Yang Y, Zhou SY, 2004. Proteome analysis of apoptotic K562 cells induced by harringtonine. *Chin J Hematol* 25(6): 323-327.

McAinsh MR, Pittman JK, 2009. Shaping the calcium signature. *New Phytol* 181(2): 275-294.

Mi SL, Fan HZ, Sun AJ, Wang KQ, Zhou YZ, Ge JB, Yang PY, 2006. Comparative proteomics of myocardial mitochondrial proteins in aging rats. *Chin J Pathophysiol* 2(12): 2306-2310.

Mishra S, Murphy LC, Nyomba BL, Murphy LJ, 2005. Prohibitin: A potential target for new therapeutics. *Trends Mol Med* 11(4): 192-197.

Podgoreanu MV, Michelotti GA, Sato Y, Smith MP, Lin S, Morris RW, Grocott HP, Mathew JP, Schwinn DA, 2005. Differential cardiac gene expression during cardiopulmonary bypass: Ischemia-independent upregulation of proinflammatory genes. *J Thorac Cardiovasc Surg* 130(2): 330-339.

Saridaki A, Panayotou G, 2005. Identification of growth factor-regulated proteins using 2D electrophoresis and mass spectrometry. *Growth Factors* 23(3): 223-232.

Schäfer U, Kurz T, Jain D, Hartmann F, Dendorfer A, Tölg R, Raasch W, Dominiak P, Katus H, Richardt G, 2002. Impaired coronary flow and left ventricular dysfunction after mechanical recanalization in acute myocardial infarction: Role of

neurohumoral activation. *Basic Res Cardiol* 97(5): 399-408.

Sodha NR, Clements RT, Feng J, Liu Y, Bianchi C, Horvath EM, Szabo C, Sellke FW, 2008. The effects of therapeutic sulfide on myocardial apoptosis in response to ischemia-reperfusion injury. *Eur J Cardiothorac Surg* 33(5): 906-913.

Sun XG, Jia YH, Chen YY, 2000. Ding Xin recipe and tanshinone ⅡA attenuates ischemia-reperfusion arrhythmia and the mechanism of nitric oxide. *Shangdong J Tradit Chin Med* 19(16): 358-360.

Sun Y, 2007. Oxidative stress and cardiac repair/remodeling following infarction. *Am J Med Sci* 334(3): 197-205.

Tang F, Wu X, Wang T, Wang P, Li R, Zhang H, Gao J, Chen S, Bao L, Huang H, Liu P, 2007. Tanshinone ⅡA attenuates atherosclerotic calcification in rat model by inhibition of oxidative stress. *Vasc Pharmacol* 46: 427-438.

Theiss AL, Obertone TS, Merlin D, Sitaraman SV, 2007. Interleukin-6 transcriptionally regulates prohibitin expression in intestinal epithelial cells. *J Biol Chem* 282(17): 12804-12812.

Wang JT, Peng DY, Liu JQ, Jin CS, 2008. Conparision of the content of tanshinone ⅡA in *Salvia miltiorrhiza* from different habitats and different organs. *Chin J Exp Tradit Med Form* 14(3): 4-5.

Winter A, Kämäräinen O, Hofmann A, 2007. Molecular modeling of prohibitin domains. *Proteins* 68(1): 353-362.

Zhang M, Zhang BL, Gao XM, Guo LP, Du R, 2003. Effect of different proportions of salvianolic acid B and tanshinone ⅡA on rat cardiomyocyte injured by tumor necrosis factor alpha *in vitro*. *Chin Pharm Bull* 19(9): 992-994.

Zhang M, Zhang BL, Gao XM, Guo LP, Du R, 2004. Effect of different proportions of salvianolic acid B and tanshinone ⅡA on rat cardiac microvascular endothelial cells injured by tumor necrosis factor α *in vitro*. *Chin Tradit Herb Drugs* 35(1): 63-65.

Editors-in-chief of Tianjin Press of Chinese Herbal Medicines Finished Training Courses by China Association for Science and Technology

Three persons in charge of Tianjin Press of Chinese Herbal Medicines, including Prof. TANG Li-da, the president of Tianjin Press of Chinese Herbal Medicines, editor-in-chief of *Chinese Traditional and Herbal Drugs* and *Drug Evaluation Research*, and associate editor-in-chief of *Chinese Herbal Medicines*, associated with manager of Tianjin Press of Chinese Herbal Medicines, Prof. CHEN Chang-qing, executive editor-in-chief of *Chinese Traditional and Herbal Drugs*, and associate editor-in-chief of *Drugs & Clinic* and *Drug Evaluation Research*, editorial director of *Chinese Herbal Medicines*, and Prof. ZOU Mei-xiang, editor-in-chief of *Drug & Clinic*, took the editor-in-chief training courses held by China Association for Science and Technology in Beijing during June 28–July 2, 2010. The three editors-in-chief systematically studied the news publishing system and periodical publishing regulations, polices on digital publication, and publication and management of sci-tech periodical. And also every one of them wrote an article on how to be a good editor-in-chief and have been certificated by General Administration of Press and Publication of the People's Republic of China.

Tianjin Press of Chinese Herbal Medicines