AUTHOR QUERY FORM

ELSEVIER	**Journal: IJCA** **Article Number: 13130**	**Please e-mail or fax your responses and any corrections to:** **E-mail: corrections.esch@elsevier.spitech.com** **Fax: +1 619 699 6721**

Dear Author,

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the 'Q' link to go to the location in the proof.

Location in article	Query / Remark: click on the Q link to go Please insert your reply or correction at the corresponding line in the proof
Q1	Please provide the following: volume number, issue details, and pagination information here.

Thank you for your assistance.

International Journal of Cardiology xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Tanshinone IIA prevents doxorubicin-induced cardiomyocyte apoptosis through Akt-dependent pathway

Hong-Jye Hong [a], Ju-Chi Liu [b], Po-Yuan Chen [c], Jin-Jer Chen [d,e,f], Paul Chan [b,1], Tzu-Hurng Cheng [c,*,1]

[a] School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan, ROC
[b] Department of Medicine, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan, ROC
[c] Department of Biological Science and Technology, College of Life Sciences, China Medical University, Taichung, Taiwan, ROC
[d] Division of Cardiology, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan, ROC
[e] Graduate Institute of Clinical Medical Science, College of Medicine, China Medical University, Taichung, Taiwan, ROC
[f] Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC

ARTICLE INFO

Article history:
Received 15 June 2010
Received in revised form 10 October 2010
Accepted 4 December 2010
Available online xxxx

Keywords:
Doxorubicin
Tanshinone IIA
Traditional Chinese medicine
Cardiomyocyte apoptosis
Akt

ABSTRACT

Background: Doxorubicin, one of the original anthracyclines, remains among the most effective anticancer drugs ever developed. Clinical use of doxorubicin is, however, greatly limited by its serious adverse cardiac effects that may ultimately lead to cardiomyopathy and heart failure. Tanshinone IIA is the main effective component of Salvia miltiorrhiza known as 'Danshen' in traditional Chinese medicine for treating cardiovascular disorders. The objective of this study was set to evaluate the protective effect of tanshinone IIA on doxorubicin-induced cardiomyocyte apoptosis, and to explore its intracellular mechanism(s).
Methods: Primary cultured neonatal rat cardiomyocytes were treated with the vehicle, doxorubicin (1 μM), tanshinone IIA (0.1, 0.3, 1 and 3 μM), or tanshinone IIA plus doxorubicin.
Results: We found that tanshinone IIA (1 and 3 μM) inhibited doxorubicin-induced reactive oxygen species generation, reduced the quantity of cleaved caspase-3 and cytosol cytochrome c, and increased Bc*L*-x_L expression, resulting in protecting cardiomyocytes from doxorubicin-induced apoptosis. In addition, Akt phosphorylation was enhanced by tanshinone IIA treatment in cardiomyocytes. The wortmannin (100 nM), LY294002 (10 nM), and siRNA transfection for Akt significantly reduced tanshinone IIA-induced protective effect.
Conclusions: These findings suggest that tanshinone IIA protects cardiomyocytes from doxorubicin-induced apoptosis in part through Akt-signaling pathways, which may potentially protect the heart from the severe toxicity of doxorubicin.

1. Introduction

Doxorubicin, one of the original anthracyclines and first isolated in the early 1960s, remains among the most effective anticancer drugs ever developed [1]. Clinical use of doxorubicin is, however, greatly limited by its serious adverse cardiac effects that may ultimately lead to cardiomyopathy and heart failure [2]. Among the various mechanisms suggested to mediate doxorubicin's cardiotoxicity, the increased formation of reactive oxygen species (ROS) [3] which ultimately results in cardiomyocyte apoptosis (or programmed cell death) is one of the most plausible [4]. Nevertheless, to date, researchers/scientists have tried out a variety of approaches aimed at preventing or mitigating the deleterious action of doxorubicin, but so far, the ability of these treatments to protect the heart from damage is limited [5]. Therefore, the development of therapies with which to prevent and/or treat the doxorubicin's cardiotoxicity remains a critical issue in both cardiology and oncology.

Tanshinone IIA, extracted from Danshen, a popular medicinal herbs used in traditional Chinese medicine, exhibits a variety of cardiovascular activities including vasorelaxation and cardio-protective effects [6–9]. However, the pretreatment effects and mechanisms of tanshinone IIA on cardio-protections are not well understood. Akt is known to regulate many survival pathways of the cardiac cells [10]; and has been reported to preserve cardiac function and prevent cardiac injury [11]. Therefore, the present study was set to evaluate the protective effect of tanshinone IIA on doxorubicin-induced cardiomyocyte apoptosis, and to identify whether the underlying mechanisms are associated with the Akt-dependent pathway.

* Corresponding author. Tel.: +886 4 22053366x2515; fax: +886 4 22071500.
E-mail addresses: chanpaul@wanfang.gov.tw (P. Chan), thcheng@mail.cmu.edu.tw (T.-H. Cheng).
[1] These authors codirected the project and contributed equally to the work.

0167-5273/$ – see front matter © 2010 Published by Elsevier Ireland Ltd.
doi:10.1016/j.ijcard.2010.12.012

2. Methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, and tissue culture reagents were purchased from Invitrogen Corporation (Carlsbad, CA, USA). 5(6)-carboxy-2′, 7′-dichlorofluorescein diacetate (DCFH-DA) was from Molecular Probes Inc. (OR, USA). All other chemicals of reagent grade were obtained from Sigma-Aldrich chemical Co. (St. Louis, MO, USA). Antibodies were purchased from Lab Frontier Co. Ltd., Seoul, Korea (anti-GAPDH), Cell Signaling Technology, Inc., Danvers, MA, USA (anti-caspase-3, anti-Ser473 phospho-Akt, anti-Akt), and Santa Cruz Biotechnology, Santa Cruz, CA, USA (anti-cytochrome *c*, anti-BcL-x_L). Tanshinone IIA (purchased from Santa Cruz Biotechnology) was dissolved in dimethyl sulfoxide (DMSO), and the DMSO content in all groups was 0.1%.

2.2. Cell culture

Primary cultures of neonatal rat cardiomyocytes were prepared as previously described [12]. The research was conducted in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health, and approved by the Institutional Animal Care and Use Committee of China Medical University (LAC-94-0069). Myocyte cultures obtained were >95% pure as revealed by immunofluorescence microscopy with counting of all nuclei [stained by 4'-6-diamidino-2-phenyindole (DAPI) (Sigma-Aldrich)] and of cells that stain positive for α-actinin (Sigma-Aldrich). The culture medium was replaced after 24 h with serum-free medium consisting of DMEM, transferring (10 μg/ml), insulin (10 μg/ml), and BrdU (0.1 mM) and exposed to agents as indicated.

2.3. TUNEL assay

Doxorubicin-mediated apoptosis in cardiomyocytes was detected with enzymatic labeling of DNA strand breaks which were identified with using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) stain by a Cell Death Detection kit (Roche, Mannheim, Germany) according to the manufacturer's directions. The apoptotic ratio was measured by flow cytometry according to the manufacturer's instructions.

2.4. Caspase-3 activity assay

In the present caspase-3 activity assay, the caspase-3 substrate rhodamine-110 (Z-DEVD-R110) was used as a prefluorescent substrate. Activity of the caspase-3 was determined using a commercial kit (Promega; Madison, WI, USA) according to the manufacturer's instructions. Briefly, after 12-h treatments with doxorubicin, tanshinone IIA, doxorubicin plus tanshinone IIA, or vehicle, caspase-3 reagent was added and incubated for 10 h. Levels of release of rhodamine-110 were measured with a luminescence spectrometer LS55 (Perkin-Elmer) at an excitation wavelength of 499 nm and an emission wavelength of 521 nm.

2.5. Western blot analysis

Western blot analysis was performed as previously described [13]. Membranes were blocked in 10 mM Tris (pH 7.5), 100 mM NaCl, and 0.1% Tween 20 containing 5% nonfat dry milk, followed by incubation with primary antibody. Membranes were washed three times and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (1:5000 dilutions) to detect bands by enhanced chemiluminescence (Amersham Biosciences Corp, NJ, USA).

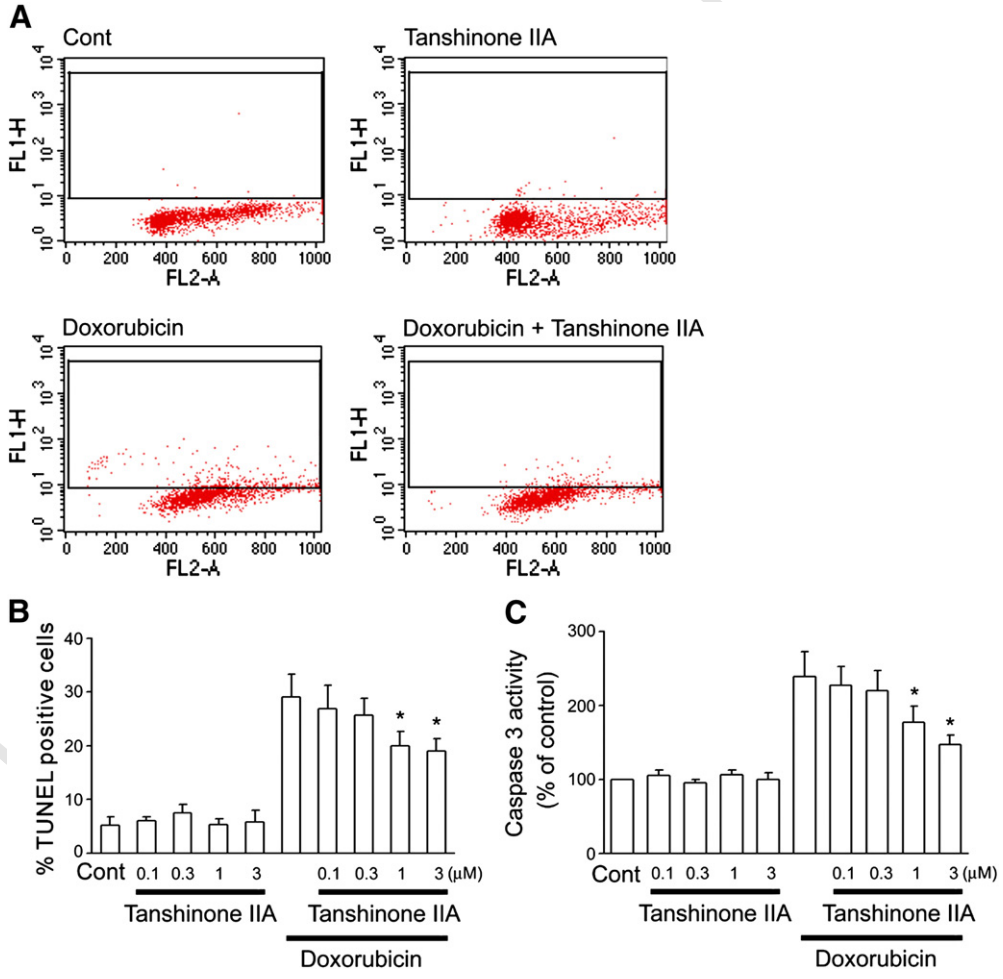

Fig. 1. Tanshinone IIA protected cardiomyocytes from doxorubicin-induced apoptosis. Results were shown in mean ± S.E.M (n = 6). *P<0.05 vs. control (Cont); #P<0.05 vs. doxorubicin. (A) Flow cytometric analysis of TUNEL-stained cells in different groups. *Cont*: control cells; *Doxorubicin*: doxorubicin-treated cells; *Tanshinone IIA*: cells treated with 3 μM tanshinone IIA; *Tanshinone IIA + Doxorubicin*: cells treated with 3 μM tanshinone IIA and doxorubicin, respectively. (B) Cardiomyocytes pretreated with tanshinone IIA (0.1, 0.3, 1, 3 μM; for 30 min) in the absence or the presence of 1 μM of doxorubicin for 24 h. Percentages of apoptotic cardiomyocytes in the different groups. (C) Cardiomyocytes pretreated with tanshinone IIA (0.1, 0.3, 1, and 3 μM; for 30 min) in the absence or the presence of 1 μM of doxorubicin for 12 h. Bars indicate the intensity of rhodamine-110 from six independent experiments, each in triplicate measurements.

Please cite this article as: Hong H-J, et al, Tanshinone IIA prevents doxorubicin-induced cardiomyocyte apoptosis through Akt-dependent pathway, Int J Cardiol (2010), doi:10.1016/j.ijcard.2010.12.012

2.6. Flow cytometric assay of 2′,7′-dichlorodihydrofluorescein oxidation

The determination of intracellular ROS production was based on the oxidation of 2′,7′-dichlorodihydrofluorescein (DCFH) to fluorescent 2′,7′-dichlorofluorescein (DCF), as described previously [14]. DCFH was added at a final concentration of 10 μM and incubated for 30 min at 37 °C. The cells were then washed once with PBS and maintained in a 1-ml culture medium. Following drug treatment, the medium was aspirated and cells were washed twice with PBS, and then dissociated with trypsin. Cellular fluorescence was determined by flow cytometry (FACS-SCAN, Becton-Dickinson, Franklin Lakes, NJ, USA). Cells were excited with an argon laser at 488 nM, and measurements were taken at 510–540 nm.

2.7. Short interfering RNA (siRNA) transfection

Akt siRNA were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA). Akt siRNAs and mock control oligonucleotides were transfected using the Lipofectamine reagent according to the manufacturer's instructions. The final concentration of Akt siRNAs for transfection was 100 nM. We washed transfected cells and incubated them in new culture media and exposed to agents as indicated.

2.8. Statistical analysis

Results are expressed as mean ± S.E.M. Statistical analysis was performed using Student's t test or analysis of variance (ANOVA) using Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA). A value of $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of tanshinone IIA on doxorubicin-induced cardiomyocyte apoptosis

By measuring the percentage of TUNEL-labeled cells with flow cytometric analysis, treatment with doxorubicin (1 μM) for 24 h increased the percentage of apoptotic cells (Fig. 1A). Tanshinone IIA treatment alone did not affect normal cell survival. In contrast, administration of tanshinone IIA to doxorubicin-treated cells was shown to prevent doxorubicin-induced cell death. A decrease in the percentage of cell apoptosis was observed in cells treated with both doxorubicin and tanshinone IIA (Fig. 1A). The pretreatment of tanshinone IIA markedly decreased the number of apoptotic cells increased by doxorubicin in a dose-dependent manner (Fig. 1B). Recent work has supported a central role for caspase family members, especially caspase-3, as effectors of apoptosis [15]. To examine whether tanshinone IIA attenuates the apoptosis induced by doxorubicin, we measured the caspase 3 activity in cells pretreated with tanshinone IIA. As shown in Fig. 1C, caspase-3 activity in doxorubicin-treated cells (1 μM; 12 h) was significantly increased compared with vehicle-treated cells. Cardiomyocytes pretreated with tanshinone IIA (1, 3 μM) for 30 min, and then additionally treated with 1 μM of doxorubicin for 12 h, significantly inhibited the activation of caspase 3 by doxorubicin (Fig. 1C).

The influence of tanshinone IIA on apoptotic markers, such as cleaved caspase, cytochrome c and Bcl-x_L, was further evaluated by western blotting analysis (Fig. 2A). As shown in Fig. 2B and C, the cleaved caspase-3 and cytosol cytochrome *c* were greatly elevated in the cells treated with 1 μM of doxorubicin for 12 h. Pretreatment with tanshinone IIA at 1 or 3 μM significantly reduced the quantity of cleaved caspase-3 and cytosol cytochrome *c*, as compared with that in doxorubicin-treated alone cells. Contrariwise, the expression of Bcl-x_L was reduced by doxorubicin treatment, which was also recovered by tanshinone IIA pretreatment (Fig. 2D). These results indicate that the pretreatment of tanshinone IIA inhibited doxorubicin-induced variations of apoptotic markers in a dose-dependent manner.

3.2. The influence of tanshinone IIA on doxorubicin-induced ROS generation in cardiomyocytes

To evaluate the mechanism of the protective effect of tanshinone IIA on doxorubicin-induced apoptosis, the influence of tanshinone IIA

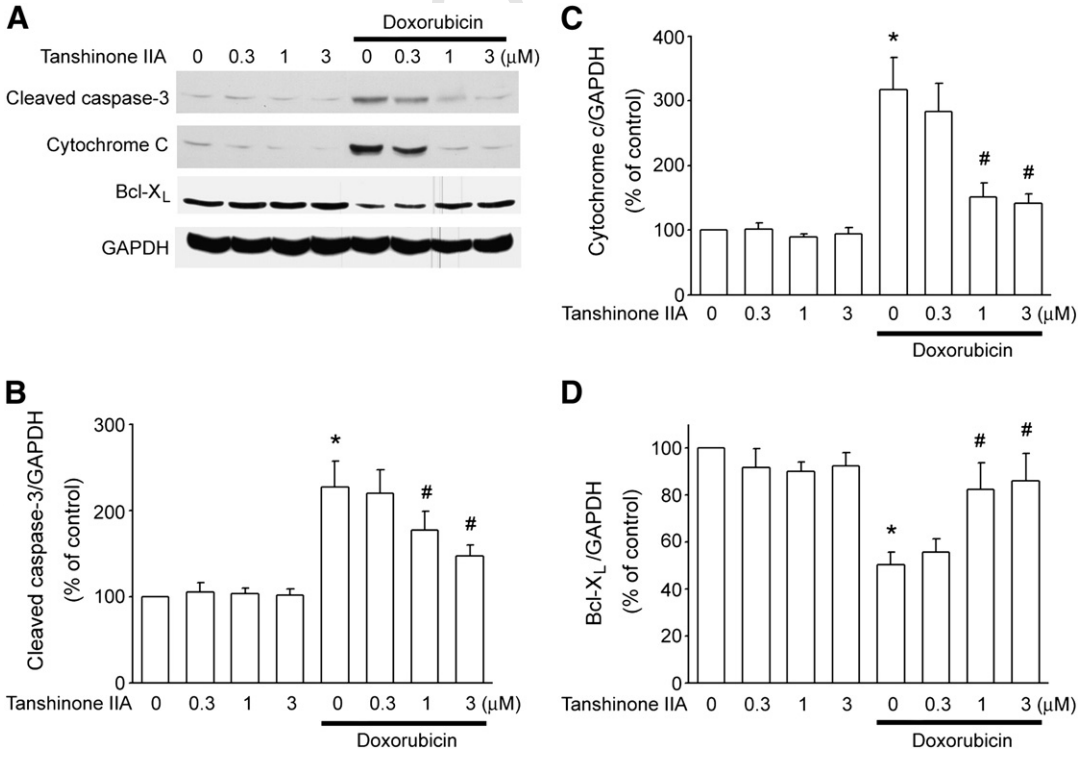

Fig. 2. Effects of tanshinone IIA on apoptotic markers (cleaved caspase-3, released cytochrome *c*, and BcL-xL) in doxorubicin-treated cardiomyocytes. Values shown were corrected using the density of GAPDH. Results were shown in mean ± S.E.M (n = 6). *$P<0.05$ vs. control (Cont); #$P<0.05$ vs. doxorubicin. (A) The cells were pretreated with tanshinone IIA (0.3, 1, and 3 μM) for 30 min, and then treated with 1 μM of doxorubicin for 12 h. Western blotting was carried out with the specific antibody against cleaved caspase-3, cytochrome c and Bcl-x_L. GAPDH was used as a loading control. Representative photomicrographs are shown. (B) Densitometric analysis of cleaved caspase-3. (C) Densitometric analysis of cytochrome *c* release. (D) Densitometric analysis of BcL-x_L.

Please cite this article as: Hong H-J, et al, Tanshinone IIA prevents doxorubicin-induced cardiomyocyte apoptosis through Akt-dependent pathway, Int J Cardiol (2010), doi:10.1016/j.ijcard.2010.12.012

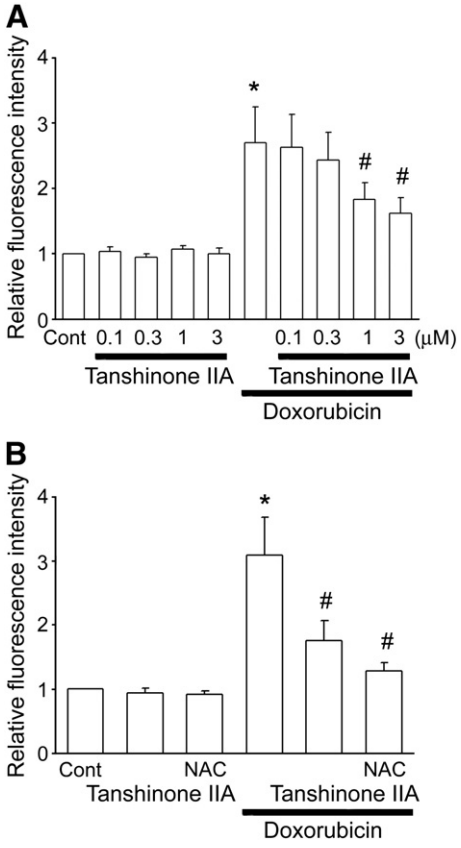

Fig. 3. Effects of tanshinone IIA on doxorubicin-induced ROS generation in cardiomyocytes. Relative fluorescence intensity in rat cardiomyocytes was quantified by flow cytometry using DCFH-DA. The fluorescence intensities in untreated control cells are expressed as 100%. Data were presented as relative intensity of experimental groups compared to untreated control cells. Results were shown in mean ± S.E.M. ($n = 6$). *P<0.05 vs. control (Cont); #P<0.05 vs. doxorubicin. (A) Column bar graph of mean cell fluorescence for DCF evaluated for cardiomyocytes pretreated with tanshinone IIA (0.1, 0.3, 1, and 3 μM; for 30 min) and thereafter in the absence or the presence of 1 μM of doxorubicin for 1 h. (B) Cells were incubated with tanshinone IIA (3 μM) or NAC (5 mM) and thereafter in the absence or the presence of 1 μM of doxorubicin for 1 h.

on doxorubicin-induced ROS generation was monitored. We examined whether tanshinone IIA prevents doxorubicin-induced ROS formation. Tanshinone IIA-pretreated cells were treated with 1 μM of doxorubicin for 1 h. Doxorubicin-induced increases in intracellular ROS were revealed by fluorescent intensities of DCF. As shown in Fig. 3A and B, tanshinone IIA or the ROS scavenger *N*-acetylcysteine (NAC; 5 mM) pretreatment significantly inhibited doxorubicin-induced ROS formation. These results indicate that the pretreatment of tanshinone IIA inhibited doxorubicin-induced ROS generation in cardiomyocytes.

3.3. Effects of tanshinone IIA on phospho-Akt in cardiomyocytes

Akt is known to have an inhibitory effect on apoptosis in several cell types including cardiomyocytes [11]. To determine the effects of tanshinone IIA on Akt phosphorylation in rat cardiomyocytes, phospho-Akt (for serine 473) was detected. As shown in Fig. 4A and B, tanshinone IIA increased serine phosphorylation of Akt from 5 to 60 min in a dose-dependent manner in cardiomyocytes. Since Akt is one of the downstream effectors of PI3K, we next examined the effects of PI3K inhibitors on Akt phosphorylation. Pretreatment with the PI3K inhibitors wortmannin (100 nM) and LY294002 (10 nM) inhibited tanshinone IIA-increased serine phosphorylation of Akt (Fig. 4C). These findings indicate that tanshinone IIA induces Akt phosphorylation *via* the PI3K/Akt pathway. Furthermore, to examine whether doxorubicin modulates Akt activity, we analysed the effects of doxorubicin on Akt phosphorylation. We examined the effects of doxorubicin (1 μM) on Akt phosphorylation at different time points, 0.5, 1, 3, and 12 h after treatment. Doxorubicin treatment produced a decline in Akt phosphorylation to below the basal levels (Fig. 4D), but these were restored to above basal levels in the cells pretreated with tanshinone IIA (Fig. 4E). To determine whether the restoration of Akt phosphorylation by tanshinone IIA is involved in the signaling of PI3K, the effect of its specific inhibitors, wortmannin and LY294002, on Akt activation was examined. Tanshinone IIA-induced restoration of Akt phosphorylation was completely inhibited by wortmannin (100 nM) and LY294002 (10 nM) (Fig. 4E). Doxorubicin-induced oxidative stress was attenuated by a free radical scavenger NAC, we also examined the effects of NAC on Akt phosphorylation in the presence of doxorubicin or anshinone IIA. However, no differences were observed in Akt phosphorylation between NAC-treated and non-treated cells in the presence of doxorubicin or tanshinone IIA (Fig. 4F).

3.4. Role of Akt in the protective effect of tanshinone IIA on doxorubicin–induced cardiomyocyte apoptosis

Finally, to identify the signaling pathways involved in the effect of tanshinone IIA, the siRNA for Akt, which mitigates the kinase activity of Akt, was applied in cardiomyocytes. The Akt protein levels were obviously reduced by Akt siRNA transfection (data not shown). The inhibitory effect of tanshinone IIA on the doxorubicin-induced caspase-3 activation was partially reversed by wortmannin (Wort; 100 nM), LY294002 (LY; 10 nM), and Akt siRNA transfection (Fig. 5A). Similarly, the inhibitory effect of tanshinone IIA on the doxorubicin-induced cardiomyocyte apoptosis was also reduced by wortmannin (Wort; 100 nM), LY294002 (LY; 10 nM), and Akt siRNA transfection (Fig. 5B). These results revealed the involvement of Akt signaling pathway in tanshinone IIA's effect on doxorubicin-induced cardiomyocyte apoptosis.

4. Discussion

The results of this study indicate that apoptosis in cardiomyocytes induced by doxorubicin can be considerably reduced by tanshinone IIA. This mechanism involves inhibiting apoptosis-related increase of ROS, activation of caspase-3, cytochrom c release, and increasing the expression of Bc*L*-x_L. We also found that tanshinone IIA upregulated Akt phosphorylation, an interesting self-gain signaling that may possibly magnify the effect of tanshinone IIA. The causal relationship between upregulated Akt phosphorylation and tanshinone IIA action, however, needs further investigations.

The results of our study demonstrated that a statistically significant reduction of TUNEL-positive cardiomyocytes was observed when tanshinone IIA was added to doxorubicin-treated cells. We also found that caspase-3 activity of cardiomyocytes is significantly increased when cells were treated with doxorubicin and that tanshinone IIA (3 μM) greatly reduced this activation. Bc*L*-x_L play important roles in apoptotic cell death, whereas caspase-3 is a key downstream effector of apoptosis. To investigate the underlying mechanism(s) of the antiapoptotic effect of tanshinone IIA, we examined expression of Bc*L*-x_L, and caspase-3. The results showed that tanshinone IIA inhibited the expression of Bax and increased the expression of Bcl-2 in cardiomyocytes. These observations suggest that tanshinone IIA may modify the imbalance of Bax and Bcl-2 in apoptotic cardiac cells. The expression of Bax, Bcl-2, and caspase-3 is consistent with the results obtained by flow cytometry with TUNEL stain.

Tanshinone IIA is the main effective component of Salvia miltiorrhiza known as 'Danshen' in traditional Chinese herbs. Clinical evidence has shown that tanshinone IIA increases coronary blood flow and protects heart against cardiac injury [16]. On the basis of the

Please cite this article as: Hong H-J, et al, Tanshinone IIA prevents doxorubicin-induced cardiomyocyte apoptosis through Akt-dependent pathway, Int J Cardiol (2010), doi:10.1016/j.ijcard.2010.12.012

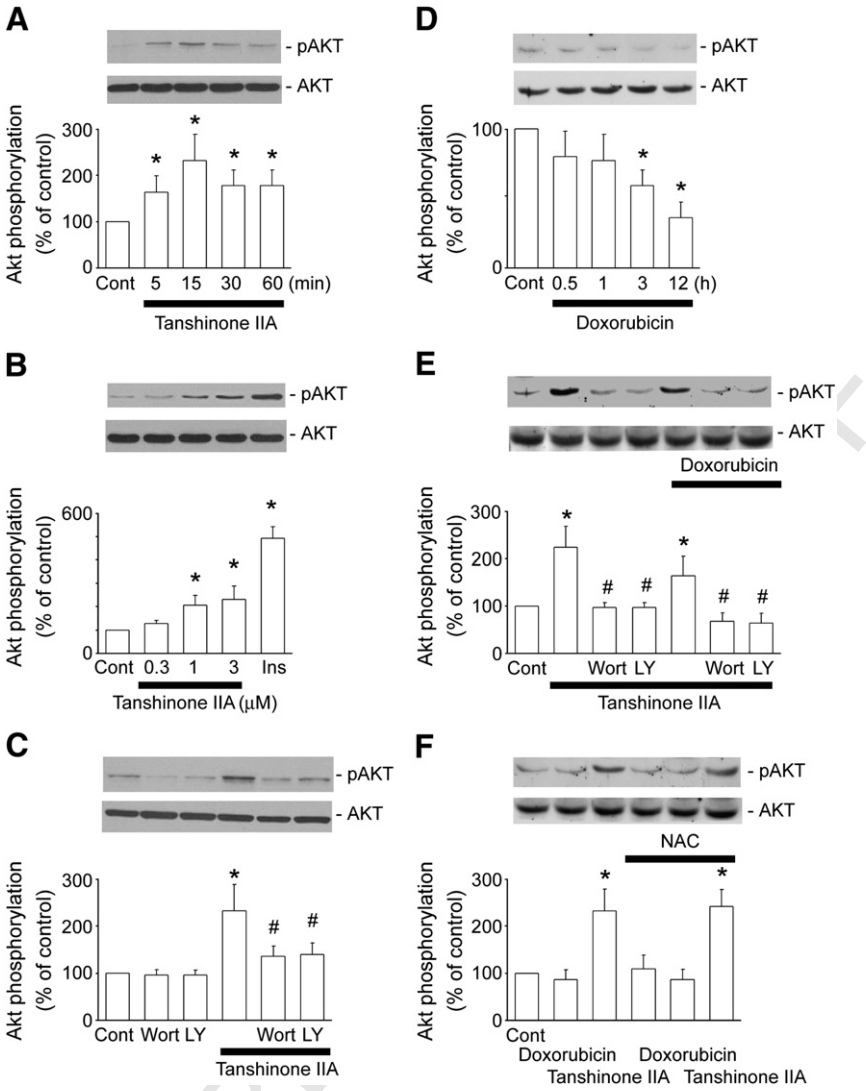

Fig. 4. Tanshinone IIA induces Akt phosphorylation *via* PI3K. (A) Neonatal cardiomyocytes were incubated with 3 μM tanshinone IIA for the indicated times. (B) Neonatal cardiomyocytes were incubated with the indicated doses of tanshinone IIA for 15 min or insulin (Ins; 100 nM, as a positive control). (C) Neonatal cardiomyocytes were incubated with wortmannin (Wort; 100 nM) or LY294002 (LY; 10 nM) for 30 min followed by incubation with 3 μM tanshinone IIA for 15 min. (D) Neonatal cardiomyocytes were incubated with 1 μM doxorubicin for the indicated times. (E) Neonatal cardiomyocytes were incubated with wortmannin (Wort; 100 nM) or LY294002 (LY; 10 nM) for 30 min followed by incubation with 3 μM tanshinone IIA in presence and absence of 1 μM doxorubicin for 15 min. (F) Neonatal cardiomyocytes were incubated with NAC (5 mM) for 30 min followed by incubation with 3 μM tanshinone IIA or 1 μM doxorubicin for 15 min. (A–E) Western blot analyses were performed using site- and phospho-specific Akt antibodies against Ser473 (p-Akt, upper blot) or total Akt (lower blot). The results are means ± SE ($n = 5$), expressed as percentage changes in phosphorylation over that in control cells. *$P<0.05$ vs. control (Cont); #$P<0.05$ vs. tanshinone IIA.

cardioprotective action of tanshinone IIA, we investigated the hypothesis that tanshinone IIA may prevent death of cardiomyocytes. Cardiomyocyte apoptosis is one of the major pathogenic mechanisms underlying myocardial injury. Blocking the apoptosis process could prevent the loss of contractile cells, minimize cardiac injury induced by injury and therefore slow down or even prevent the occurrence of heart failure [17]. Thus, we performed TUNEL staining in order to explore the underlying mechanism responsible for the cardiac function improvement induced by tanshinone IIA. The results indicated that tanshinone IIA inhibited cardiomyocyte apoptosis induced by doxorubicin which was similar to previous reports that tanshinone IIA protected cardiac myocytes against oxidative stress-triggered damage and apoptosis [7,17]. The possible mechanisms which have been proposed for the protective effects of tanshinone IIA include antioxidant properties by scavenging free radicals in cardiomyocytes [6]. In addition, Akt, a serine/threonine kinase, is a primary mediator of the downstream effects of PI3K, coordinating a variety of intracellular signals and regulating cell proliferation and survival. Recent studies have also shown that activation of the PI3K/Akt signalling pathway protects the myocardium from myocardial injury and prevents cardiac myocyte apoptosis [11]. Our data imply that the restoration of Akt phosphorylation by tanshinone IIA correlates well with cell survival. In order to explore whether the protective effects of tanshinone IIA are associated with Akt pathway, the Akt siRNA was employed to observe the effects of co-administration of Akt siRNA and tanshinone IIA compared with tanshinone IIA alone. Co-administration of Akt siRNA and tanshinone IIA enhanced cardiomyocytes apoptosis and associated with decreased p-Akt expression. In other words, the siRNA transfection for Akt can abolish the cardiac protective effects of tanshinone IIA. These results suggest that tanshinone IIA induces cardioprotective effects through the activation of Akt-pathway. The results of this study suggest that tanshinone IIA may offer a practicable approach to the reduction of apoptosis of cardiomyocytes and may merit further investigation.

Please cite this article as: Hong H-J, et al, Tanshinone IIA prevents doxorubicin-induced cardiomyocyte apoptosis through Akt-dependent pathway, Int J Cardiol (2010), doi:10.1016/j.ijcard.2010.12.012

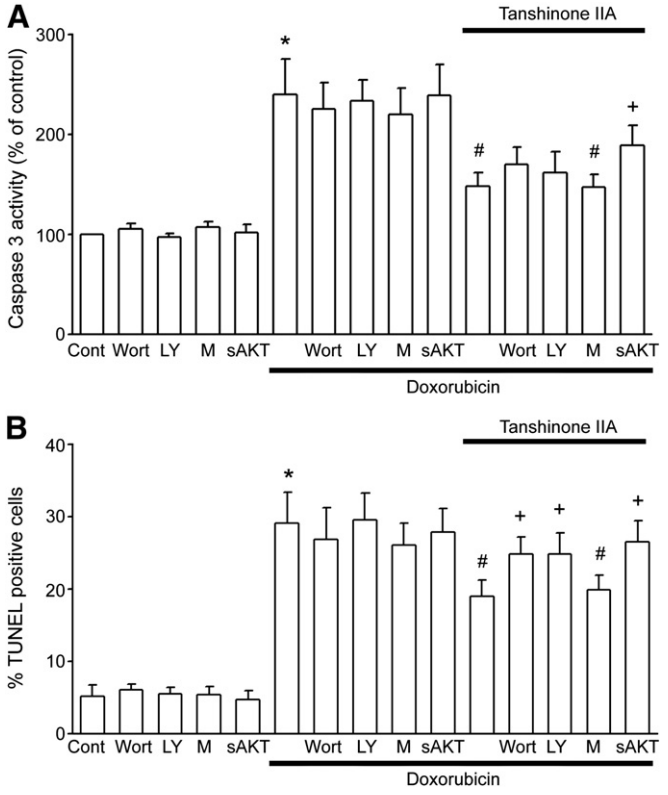

Fig. 5. Blockage of Akt pathway attenuated the inhibitory effect of tanshinone IIA on doxorubicin-induced apoptosis. Results were shown as mean ± S.E.M. (n = 6). *P<0.05 vs. the mock control; #P<0.05 vs. doxorubicin. +P<0.05 vs. tanshinone IIA plus doxorubicin. Notes: Cont, untransfected control; M, mock control; sAkt, Akt siRNA transfection. (A) The effect of wortmannin (Wort; 100 nM), LY294002 (LY; 10 nM), and Akt siRNA on tanshinone IIA-decreased doxorubicin-induced caspase-3 activity in cardiomyocytes. Transfected cells were pretreated with or without tanshinone IIA (3 μM) for 30 min, and then treated with doxorubicin (1 μM) for 12 h. (B) The effect of wortmannin (Wort; 100 nM), LY294002 (LY; 10 nM), and Akt siRNA on tanshinone IIA-decreased doxorubicin-induced apoptosis in cardiomyocytes. Transfected cells were pretreated with or without tanshinone IIA (3 μM) for 30 min, and then treated with doxorubicin (1 μM) for 24 h.

In summary, the present study demonstrates strongly that tanshinone IIA protects neonatal cardiomyocytes from doxorubicin-induced apoptosis. Tanshinone IIA might potentially be developed to treat heart failure or other apoptosis-related heart diseases if further studies were performed to define and clarify rationale for its clinical use.

Conflict of interest

None declared.

Acknowledgments

This work was supported by National Science Council Grants (NSC 96-2320-B-038-016-MY3), and Grant No CMU-98-S-22 from the China Medical University, Taichung, Taiwan, ROC [18].

References

[1] Salem PA. Advances in cancer chemotherapy. J Méd Liban 1975;28:9–24.
[2] Czarnecki A. Doxorubicin-induced cardiomyopathy. Pol Tyg Lek 1983;38:471–3.
[3] Deng S, Kruger A, Kleschyov AL, Kalinowski L, Daiber A, Wojnowski L. Gp91phox-containing NAD(P)H oxidase increases superoxide formation by doxorubicin and NADPH. Free Radic Biol Med 2007;42:466–73.
[4] Spallarossa P, Altieri P, Garibaldi S, et al. Matrix metalloproteinase-2 and -9 are induced differently by doxorubicin in H9c2 cells: the role of MAP kinases and NAD(P)H oxidase. Cardiovasc Res 2006;69:736–45.
[5] Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management. Prog Cardiovasc Dis 2007;49:330–52.
[6] Zhou L, Zuo Z, Chow MS. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol 2005;45:1345–59.
[7] Gao J, Yang G, Pi R, et al. Tanshinone IIA protects neonatal rat cardiomyocytes from adriamycin-induced apoptosis. Transl Res 2008;151:79–87.
[8] Sun DD, Wang HC, Wang XB, et al. Tanshinone IIA: a new activator of human cardiac KCNQ1/KCNE1 (I(Ks)) potassium channels. Eur J Pharmacol 2008;590:317–21.
[9] Xu W, Yang J, Wu LM. Cardioprotective effects of tanshinone IIA on myocardial ischemia injury in rats. Pharmazie 2009;64:332–6.
[10] Shiraishi I, Melendez J, Ahn Y, et al. Nuclear targeting of Akt enhances kinase activity and survival of cardiomyocytes. Circ Res 2004;94:884–91.
[11] Matsui T, Tao J, del Monte F, et al. Akt activation preserves cardiac function and prevents injury after transient cardiac ischemia in vivo. Circulation 2001;104:330–5.
[12] Cheng TH, Shih NL, Chen SY, Wang DL, Chen JJ. Reactive oxygen species modulate endothelin-1-induced c-fos gene expression in cardiomyocytes. Cardiovasc Res 1999;41:654–62.
[13] Chao HH, Liu JC, Hong HJ, Lin JW, Chen CH, Cheng TH. L-carnitine reduces doxorubicin-induced apoptosis through a prostacyclin-mediated pathway in neonatal rat cardiomyocytes. Int J Cardiol 2009. Q1
[14] Chen YL, Liu JC, Loh SH, et al. Involvement of reactive oxygen species in urotensin II-induced proliferation of cardiac fibroblasts. Eur J Pharmacol 2008;593:24–9.
[15] Boatright KM, Salvesen GS. Mechanisms of caspase activation. Curr Opin Cell Biol 2003;15:725–31.
[16] Jin UH, Suh SJ, Chang HW, et al. Tanshinone IIA from Salvia miltiorrhiza BUNGE inhibits human aortic smooth muscle cell migration and MMP-9 activity through AKT signaling pathway. J Cell Biochem 2008;104:15–26.
[17] Fu J, Huang H, Liu J, Pi R, Chen J, Liu P. Tanshinone IIA protects cardiac myocytes against oxidative stress-triggered damage and apoptosis. Eur J Pharmacol 2007;568:213–21.
[18] Shewan LG, Coats AJ. Ethics in the authorship and publishing of scientific articles. Int J Cardiol 2010;144:1–2.

Please cite this article as: Hong H-J, et al, Tanshinone IIA prevents doxorubicin-induced cardiomyocyte apoptosis through Akt-dependent pathway, Int J Cardiol (2010), doi:10.1016/j.ijcard.2010.12.012