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Tanshinone IIA Inhibits Angiotensin II-induced Cell Proliferation in Rat Cardiac Fibroblasts

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Abstract: Tanshinone IIA extracted from Danshen, a popular medicinal herb used in traditional Chinese medicine, exhibits cardio-protective effects. However, the mechanism of its cardio-protective effect is not well established. The aims of this study were to examine whether tanshinone IIA may alter angiotensin II (Ang II)-induced cell proliferation and to identify the putative underlying signaling pathways in rat cardiac fibroblasts. Cultured rat cardiac fibroblasts were pre-treated with tanshinone IIA and stimulated with Ang II, cell proliferation and endothelin-1 (ET-1) expression were examined. The effect of tanshinone IIA on Ang II-induced reactive oxygen species (ROS) formation, and extracellular signal-regulated kinase (ERK) phosphorylation were also examined. In addition, the effect of tanshinone IIA on nitric oxide (NO) production, and endothelial nitric oxide synthase (eNOS) phosphorylation were tested to elucidate the intracellular mechanism. The increased cell proliferation and ET-1 expression by Ang II (100 nM) were partially inhibited by tanshinone IIA. Tanshinone IIA also inhibited Ang II-increased ROS formation, and ERK phosphorylation. In addition, tanshinone IIA was found to increase the NO generation, and eNOS phosphorylation. N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NOS, and the short interfering RNA transfection for eNOS markedly attenuated the inhibitory effect of tanshinone IIA on Ang II-induced cell proliferation. The results suggest that tanshinone IIA prevents cardiac fibroblast proliferation by interfering with the generation of ROS and involves the activation of the eNOS-NO pathway.

Keywords: Tanshinone IIA; Traditional Chinese Medicine; Angiotensin II; Cardiac Fibroblast Proliferation; Nitric Oxide.

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Introduction

Tanshinone IIA, extracted from Danshen, a popular medicinal herb used in traditional Chinese medicine, exhibits a variety of cardiovascular activities including vasorelaxation and cardio-protective effects (Gao *et al.*, 2008; Sun *et al.*, 2008; Xu *et al.*, 2009; Zhou *et al.*, 2005). However, the pretreatment effects and mechanisms of tanshinone IIA on cardio-protection are not well understood. Cardiac fibrosis, characterized by the proliferation of cardiac fibroblasts and abundant accumulation of matrix proteins in the extracellular space, is one of the deleterious events accompanying hypertension that may participate in the progression toward heart failure (Schnee and Hsueh, 2000). Angiotensin II (Ang II), the effector peptide of the renin-angiotensin system, is a key pathogenic factor in the development of heart failure. Ang II induces cardiac myocyte hypertrophy, fibroblast proliferation, and collagen formation (Schnee and Hsueh, 2000). It is well known that Ang II stimulates reactive oxygen species (ROS) in cardiovascular tissues, which is involved in cardiac remodeling (Zafari *et al.*, 1998). We have reported that ROS are essential for Ang II-induced endothelin-1 (ET-1) gene expression and cell proliferation in cardiac fibroblasts (Cheng *et al.*, 2003). Tanshinone IIA is known to act as an antioxidant, reacting with free radicals to prevent oxidative cell damage in several types of preparations (Liu *et al.*, 2008; Yang *et al.*, 2009, 2008) Application of tanshinone IIA also stimulates the production of nitric oxide (NO) in cultured vascular endothelial cells (Huang *et al.*, 2007; Lin *et al.*, 2006; Wu *et al.*, 2009). NO derived from endothelial nitric oxide synthase (eNOS) was reported to play a key role in the regulation of cardiac fibrosis (Smith *et al.*, 2005). However, no study has addressed the effects of tanshinone IIA on cardiac fibroblast proliferation. The aims of this study were to investigate the anti-proliferative effect of tanshinone IIA on Ang II-induced cardiac fibroblast cell growth and to identify whether the underlying mechanisms are associated with the NO-dependent pathway.

Materials and Methods

Materials

Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, and tissue culture reagents were from Life Technologies, Inc (Gaithersburg, MD. USA). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) was obtained from Invitrogen (Carlsbad, CA, USA). Tanshinone IIA (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was dissolved in dimethyl sulfoxide (DMSO), and the DMSO content in all groups was 0.1%. N-nitro-L-arginine methyl ester (L-NAME) and all other reagent-grade chemicals were purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Rabbit polyclonal anti-phospho-ERK antibody and anti-ERK antibody were purchased from New England Biolabs (Beverly, MA, USA) and Santa Cruz Biotechnology Inc. Anti-Ser1177 phospho-eNOS antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA); and anti-eNOS antibodies were from BD Bioscience (San Jose, CA, USA).

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Culture of Cardiac Fibroblasts

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and was approved by the Institutional Animal Care and Use Committee of China Medical University. Primary cultures of neonatal rat cardiac fibroblasts were prepared as previously described (Cheng *et al.*, 2003). Briefly, ventricles from 1- to 2-day-old neonatal Sprague-Dawley rats were cut into chunks of approximately 1 mm^3 by using scissors and were subjected to trypsin (0.125%; Invitrogen) digestion in phosphate buffered saline (PBS). Dispersed cells were incubated on 100 mm culture dishes for 30 min in a 5% CO_2 incubator. Nonmyocytes attached to the bottom of the dishes were subsequently incubated with DMEM supplemented with 10% fetal calf serum for an additional 2 to 4 days. Confluent nonmyocytes were treated with trypsin and subcultured. Subconfluent ($\sim$70% confluency) cardiac fibroblasts grown in culture dishes from the second to fourth passage were used in the experiments and were $>$99% positive for vimentin antibodies (Sigma-Aldrich). Serum-containing medium from the cultured cells was replaced with serum-free medium, and the cells were then exposed to the agents as indicated.

Cell Proliferation

Proliferation was assessed by quantifying 5-bromo-2′-deoxyuridine (BrdU) incorporation in the presence or absence of reagents as indicated. The rate of cellular proliferation was determined by cell counting. Cells were removed from the culture dish by addition of trypsin and then centrifuged. The pellet was resuspended in 1 ml DMEM and cells were counted in an automatic cell counter (S.ST.II/ZM, Coulter Electronics Ltd., Miami, FL, USA). Cell proliferation was assessed by the incorporation of BrdU. Cells (1×10^4 cells/well) were incubated in 96-well plastic plates. Then, BrdU (10 μM) was added to the medium and the cells were incubated for another 18 h. Subsequently, the cells were fixed and BrdU incorporation was determined with a Cell Proliferation enzyme-linked immunosorbent assay (ELISA) Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

RNA Extraction and Quantitative Polymerase Chain Reaction (Q-PCR) Analysis

Total RNA was extracted from cardiac fibroblasts using the TRIzol method according to the protocol recommended by the manufacturer (Invitrogen), and used to synthesize single-stranded complementary (c)DNA with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). ET-1 mRNA was quantified using TaqMan Gene Expression Master Mix (Applied Biosystems) with specific primers in an ABI 7300 Real-Time PCR System (Applied Biosystems). TaqMan Gene Expression Assay kits containing specific primers for ET-1 (lot no. Rn00561129_m1), and GAPDH (lot no. Rn01775763_g1) were obtained from Applied Biosystems. The specific primers for GAPDH

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were used to normalize the amount of sample added. The relative quantitation of ET-1 mRNA was analyzed using the comparative Ct method. All quantifications were performed in triplicate samples for three separate experiments.

Measurement of ET-1 Concentration

ET-1 levels were measured in culture medium using a commercial enzyme-linked immunosorbent assay kit (Amersham-Pharmacia Biotech, Little Chalfont, UK). Results were normalized to cellular protein content in all experiments, and expressed as a percentage relative to the control group.

Flow Cytometric Assay of 2′,7′-dichlorodihydrofluorescin Oxidation

The determination of intracellular reactive oxygen species production was based on the oxidation of 2′,7′-dichlorodihydrofluorescin (DCFH) to a fluorescent 2′,7′-dichlorofluorescin (DCF). DCFH was added to the cells 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 the 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.

Western Blot Analysis

Western blot analysis was performed as previously described (Cheng *et al.*, 2003). Whole-cell extracts were obtained in a RIPA buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1% SDS, 1.0% Triton X-100, 1% deoxycholate, 5 mM EDTA, 1 mM sodium orthovanadate, 1 mM PMSF, and protease inhibitor cocktail; Complete, Roche Diagnostics GmbH, Germany). Extracts or proteins were separated by SDS-PAGE followed by electrotransfer to polyvinylidene difluoride (PVDF) membranes and probed with antisera, followed by horseradish peroxidase-conjugated secondary antibodies. The proteins were visualized by chemiluminescence, according to the manufacturer's instructions (Pierce Biotechnology Inc., IL, USA).

Measurement of Nitrate/Nitrite Levels

The culture medium was stored at −70°C until use. After it had been thawed, the sample was deproteined with two volumes of 4°C 99% ethanol and centrifuged (3000 g for 10 min). These medium samples (100 μl) were injected into a collection chamber containing 5% VCl_3. This strong reducing environment converts both nitrate and nitrite to nitric oxide. A constant stream of helium gas carried nitric oxide into a nitric oxide analyzer (Seivers 270B NOA; Seivers Instruments Inc., Boulder, CO, USA), where nitric oxide reacted with ozone, resulting in the emission of light. Light emission is proportional to the quantity of nitric oxide formed; standard amounts of nitrate were used for calibration.

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Short Interfering RNA (siRNA) Transfection

We purchased eNOS siRNA from Santa Cruz Biotechnology. eNOS siRNA, and mock control oligonucleotides were transfected using the Lipofectamine reagent according to the manufacturer's instructions. The final concentration of siRNAs for transfection was 100 nM. Transfected cells were washed with PBS, and then incubated in new culture media for an additional treatment as indicated.

Statistical Analysis

Results are expressed as mean $\pm$ S.E.M. for at least six experiments unless designated otherwise. Statistical analysis was performed using Student's *t* test or analysis of variance (ANOVA) followed by Tukey's multiple comparisons with the GraphPad Prism (GraphPad Software, San Diego, CA, USA). A value of $p < 0.05$ was considered to be statistically significant.

Results

Effects of Tanshinone IIA on Ang II-induced Cell Proliferation of Cardiac Fibroblasts

The effects of tanshinone IIA on Ang II-stimulated rat cardiac fibroblast proliferation were assessed by analyzing DNA synthesis with BrdU incorporation and cell counting. Pretreatment of cardiac fibroblasts with tanshinone IIA (3 and 10 μM) for 30 min followed by exposure to Ang II (100 nM) for 24 h, resulted in a significant decrease in Ang II-increased cell number and BrdU incorporation (Figs. 1A and 1B). These data clearly suggest that tanshinone IIA inhibited Ang II-induced proliferation of cardiac fibroblasts.

Effects of Tanshinone IIA on Ang II-induced ET-1 Expression in Cardiac Fibroblasts

Cardiac fibroblasts were preincubated with tanshinone IIA (0.1, 0.3, 1, 3, and 10 μM, 30 min) before exposure to Ang II (100 nM) for 30 min, and then assayed for tanshinone IIA-inhibited Ang II-induced ET-1 expression. Tanshinone IIA (3 and 10 μM) caused a down-regulation of Ang II-induced ET-1 mRNA (Fig. 2A). Exposure of cardiac fibroblasts to Ang II (100 nM) for 24 h significantly increased ET-1 peptide secretion (Fig. 2B). Pretreatment of cardiac fibroblasts with tanshinone IIA (3 and 10 μM) inhibited Ang II-induced ET-1 secretion (Fig. 2B). These data indicate that tanshinone IIA inhibited Ang II-induced ET-1 expression in cardiac fibroblasts.

Effects of Tanshinone IIA on Ang II-increased ROS Formation and ERK Phosphorylation

We previously reported that ROS were involved in the activation of the ERK pathway, which culminated in ET-1 gene expression (Cheng *et al.*, 2003). In this study, we further

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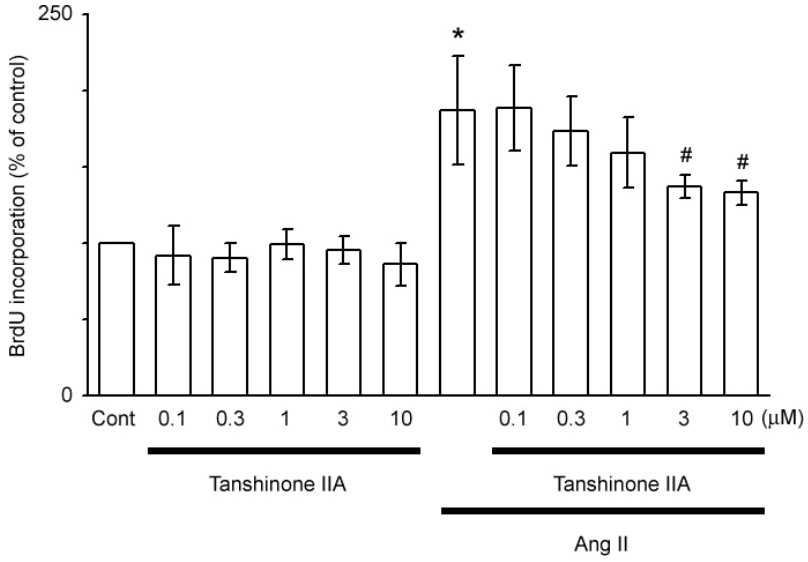
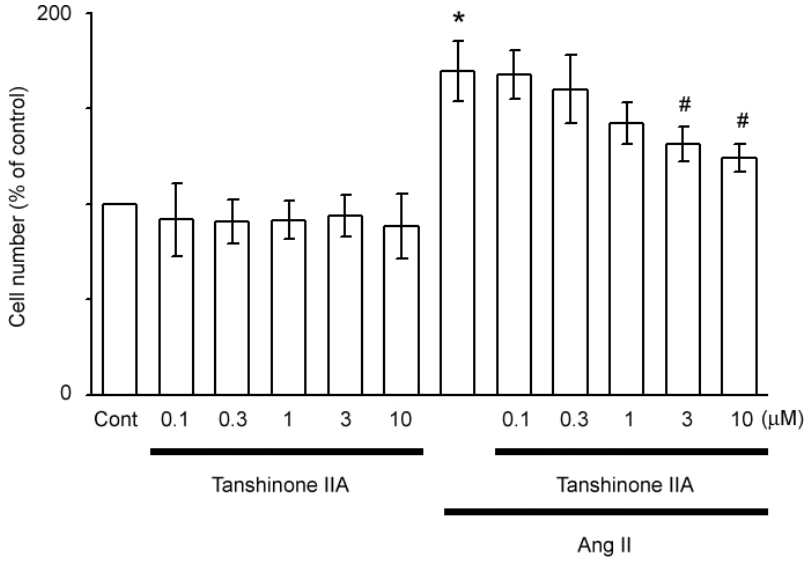


(A)



(B)

Figure 1. Effects of tanshinone IIA on the Ang II-induced cell proliferation in cardiac fibroblasts. Cells were preincubated with the indicated concentrations of tanshinone IIA and treated with Ang II (100 nM) for 24 h. Cell number and BrdU incorporation were expressed as percentage of control (Cont). Results were shown as mean ± S.E.M. ($n = 6$). *$p < 0.05$ versus control; #$p < 0.05$ versus Ang II alone. (A) Tanshinone IIA inhibits Ang II-induced DNA synthesis. Cell proliferation was estimated from the incorporation of BrdU and calculated as a percentage of the control value. (B) Tanshinone IIA inhibits Ang II-induced cell proliferation. Cells were counted for cell number and calculated as a percentage of the control value.

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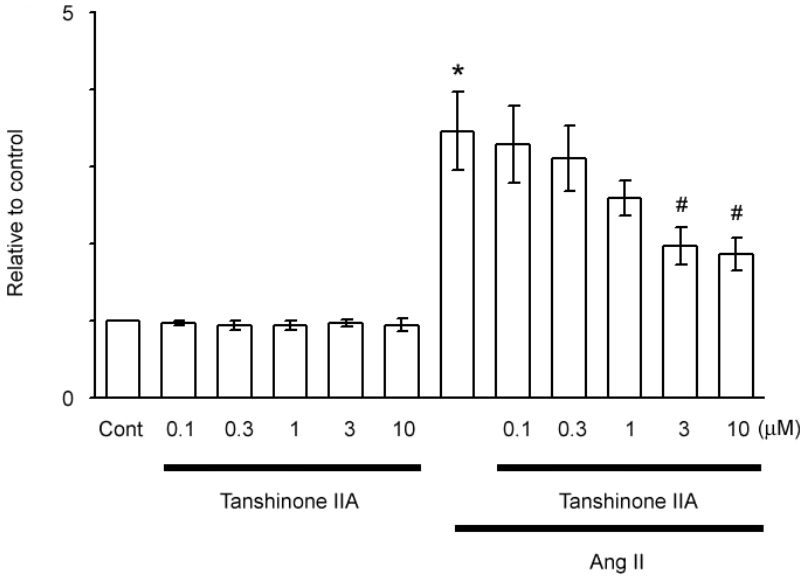

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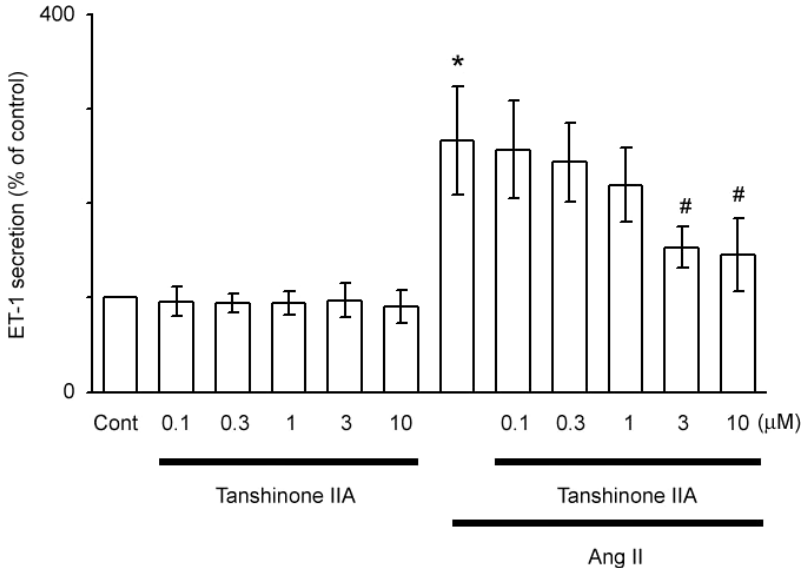

(B)

Figure 2. Tanshinone IIA down-regulates Ang II-induced ET-1 expression in cardiac fibroblasts. Results were shown as mean ± S.E.M. ($n = 6$). *p < 0.05 versus control; #p < 0.05 versus Ang II alone. (A) Down-regulation of Ang II-induced ET-1 mRNA by tanshinone IIA. Cells were preincubated with tanshinone IIA (0.1, 0.3, 1, 3, and 10 μM) and then stimulated with Ang II (100 nM) for 30 min. (B) Tanshinone IIA inhibits Ang II-induced ET-1 secretion. Cells were preincubated with tanshinone IIA (0.1, 0.3, 1, 3, and 10 μM) and then stimulated with Ang II (100 nM) for 24 h.

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examined whether tanshinone IIA prevented Ang II-increased ROS formation and ERK phosphorylation in cardiac fibroblasts. As shown in Fig. 3A, pretreatment with tanshinone IIA (3 μM) or the ROS scavenger *N*-acetylcysteine (NAC; 5 mM) significantly inhibited Ang II-induced ROS production. As shown in Fig. 3B, exposure of cardiac fibroblasts to Ang II (100 nM) for 30 min rapidly activated phosphorylation of ERK. However, cardiac fibroblasts pretreated with tanshinone IIA (3 μM, 30 min) or NAC (5 mM) had significantly decreased levels of Ang II-induced ERK phosphorylation. These findings support that tanshinone IIA inhibited Ang II-increased intracellular ROS levels and ERK signaling pathway in cardiac fibroblasts.

Effects of Tanshinone IIA on Nitric Oxide Synthesis and Phospho-eNOS in Cardiac Fibroblasts

Exposure of cardiac fibroblasts to tanshinone IIA enhanced nitric oxide generation time- and dose-dependently (Figs. 4A and 4B). In addition, tanshinone IIA treatment in cardiac fibroblasts significantly enhanced phospho-eNOS (Fig. 4C). These findings reveal that tanshinone IIA increased nitric oxide production and eNOS phosphorylation in cardiac fibroblasts.

Role of eNOS-NO Pathway in the Inhibitory Effect of Tanshinone IIA on Ang II-induced Cardiac Fibroblast Proliferation

To identify the signaling pathways involved in the effect of tanshinone IIA, a NOS inhibitor *N*-nitro-L-arginine methyl ester (L-NAME) and the short interfering RNA transfection for eNOS were applied in cardiac fibroblasts. The inhibitory effect of tanshinone IIA on the Ang II-induced ET-1 expression was partially reversed by L-NAME or the short interfering RNA transfection for eNOS (Figs. 5A and 5B). Similarly, the inhibitory effect of tanshinone IIA on Ang II-increased BrdU incorporation and cell proliferation were also reduced by L-NAME or the short interfering RNA transfection for eNOS (Figs. 5C and 5D). These results reveal the involvement of eNOS-NO signaling pathway in tanshinone IIA's effect on Ang II-induced cardiac fibroblast proliferation.

Discussion

The major finding of this study is that tanshinone IIA inhibits cardiac fibroblast proliferation by interfering with the generation of ROS and involves in the activation of the eNOS-NO pathway.

The increased levels of different humoral factors such as Ang II and ET-1 may cause the development of cardiac hypertrophy and cardiac fibrosis, leading to an enhancement of cardiac remodeling during the development of heart failure (Porter and Turner, 2009; Rehsia and Dhalla, 2010). Cardiac fibrosis is a characterization of heart disease and is the result of a variety of structural changes that occur after pathological stimuli to the cardiovascular system (Porter and Turner, 2009). Fibroblasts play a pivotal role in the development of

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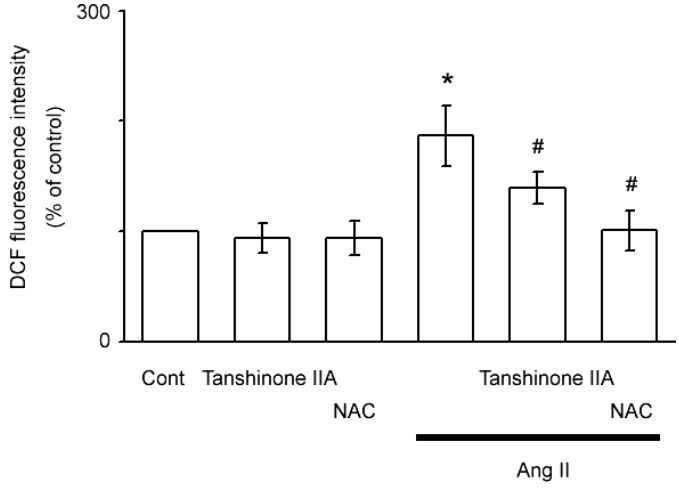
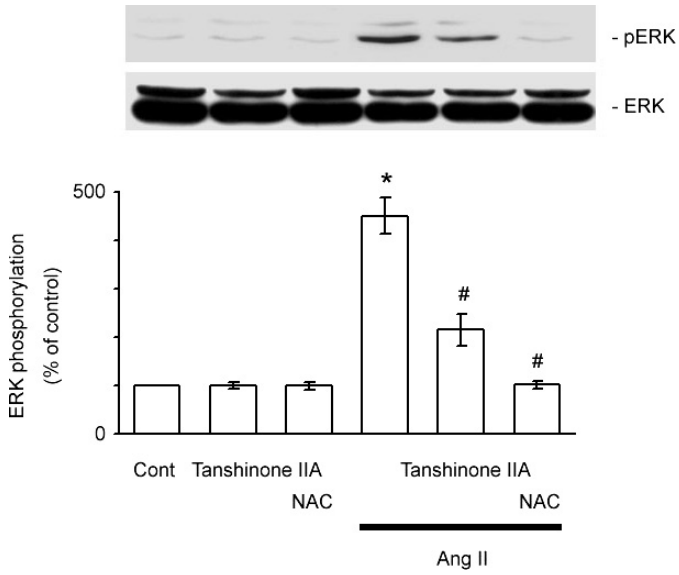


(A)



(B)

Figure 3. Effects of tanshinone IIA on Ang II-increased ROS formation and ERK phosphorylation. Cells were preincubated with tanshinone IIA (3 μM) for 30 min and then stimulated with Ang II (100 nM) for 30 min or not. *$p < 0.05$ versus control; #$p < 0.05$ versus Ang II alone. (A) Effects of tanshinone IIA on Ang II-induced ROS generation. Cardiac fibroblasts were treated with vehicle control (Cont), tanshinone IIA (3 μM) or NAC (5 mM) for 60 min, Ang II (100 nM) for 30 min, or preincubated with tanshinone IIA (3 μM) or NAC (5 mM) for 30 min and then stimulated with Ang II. Column bar graph of mean cell fluorescence for DCF, the fluorescence intensities in untreated control cells are expressed as 100%. Results were shown as mean $\pm$ S.E.M. ($n = 6$). (B) Effects of tanshinone IIA on Ang II-increased ERK phosphorylation. Cells were preincubated with tanshinone IIA (3 μM) or NAC (5 mM) and then stimulated with Ang II (100 nM) for 30 min. Phosphorylation of ERK was detected by Western blotting using anti-phospho-ERK antibody. Densitometric analyses were performed with a densitometer. Data was shown as fold increase relative to control groups. Results were shown as mean $\pm$ S.E.M. ($n = 4$).

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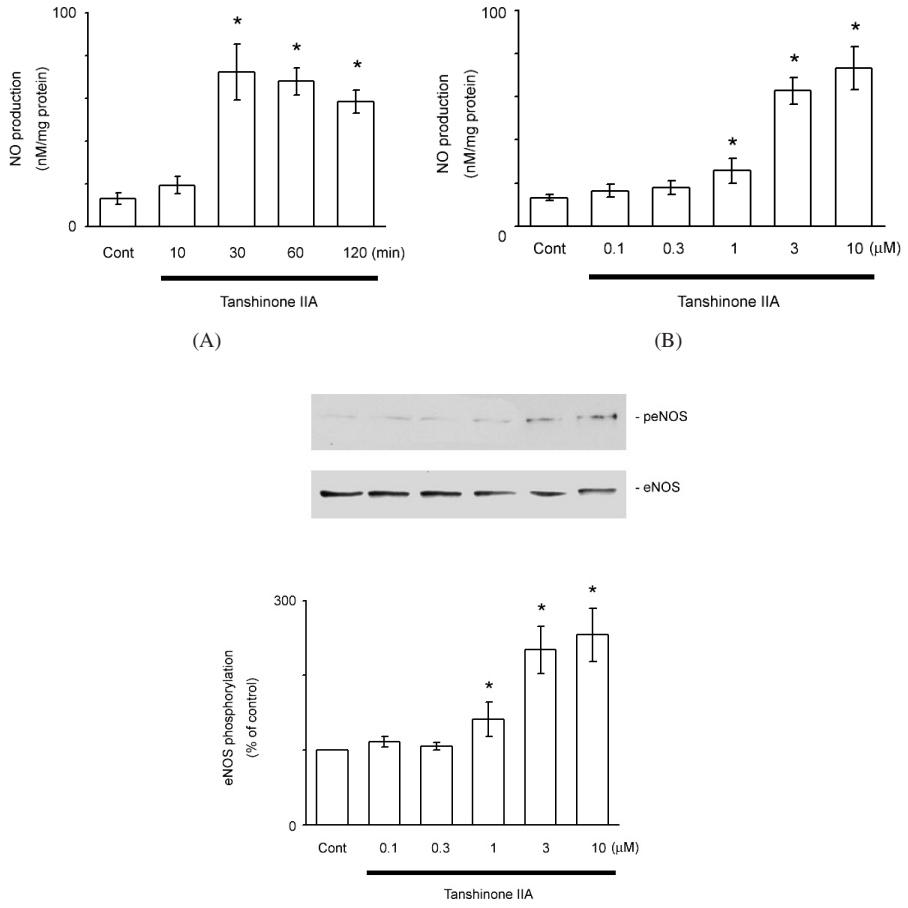

Figure 4. Effects of tanshinone IIA on NO production, and eNOS phosphorylation. *p < 0.05 versus control. (A) Time course of tanshinone IIA-induced NO production in cardiac fibroblasts. Cells were in control condition (Cont), treated with tanshinone IIA (3 μM) for 10, 30, 60, or 120 min. Results were shown as mean $\pm$ S.E.M. ($n = 5$). (B) Cells from either control or treated with tanshinone IIA (0.3, 1, 3 μM) for 30 min. Results were shown as mean $\pm$ S.E.M. ($n = 4$). (C) Effects of tanshinone IIA on phosphorylation of eNOS in cardiac fibroblasts. Western analysis performed to detect phospho-eNOS (Ser1177) and total eNOS. Results were shown as means $\pm$ S.E.M. ($n = 6$).

cardiac fibrosis, progression of left ventricular remodeling and results in diastolic dysfunction, accounting for 50% to 70% of congestive heart failure in clinical practice (Porter and Turner, 2009). Studies have shown cardioprotective roles of tanshinone IIA (Fu *et al.*, 2007; Wu *et al.*, 1993; Xu *et al.*, 2009; Yang *et al.*, 2008); however, there is no existing study addressing the interference of tanshinone IIA on ET-1 expression, ROS and NO production in cardiac fibroblasts.

In our experiments, tanshinone IIA was diluted in 0.1% DMSO. As to exclude the potential impact of emulsion, the solvent DMSO alone (0.1%) did not affect the cardiac

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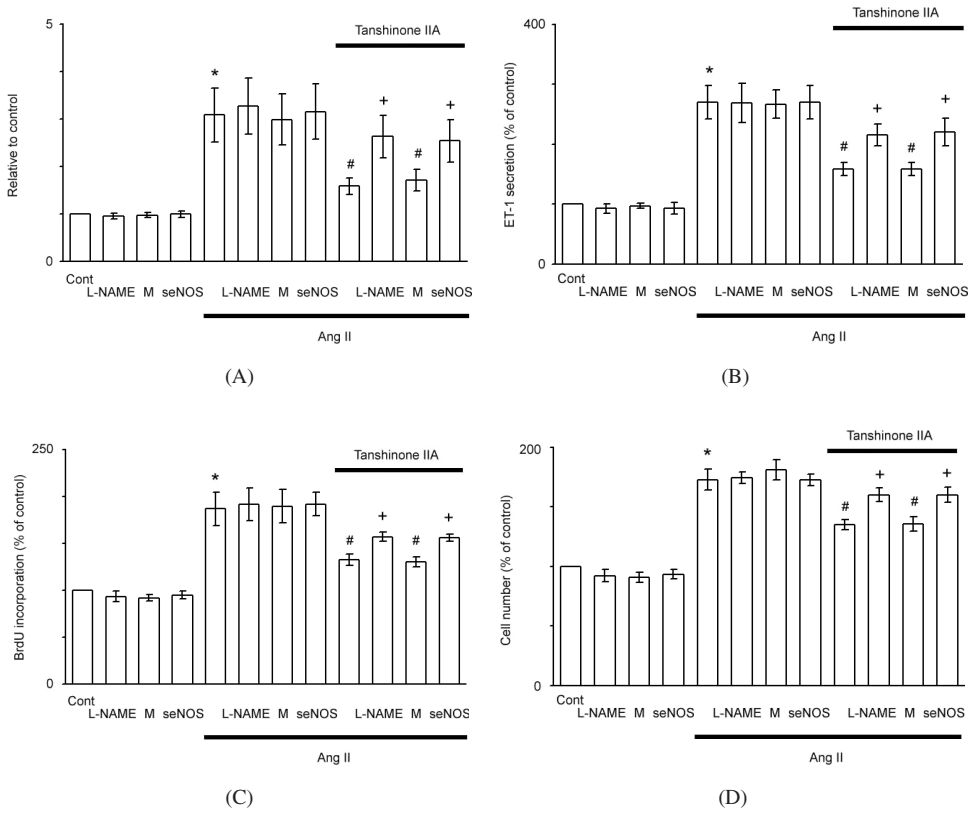

Figure 5. Blockage of eNOS-NO pathway attenuated the inhibitory effect of tanshinone IIA on Ang II-induced ET-1 expression and cell proliferation in cardiac fibroblasts. For inhibiting the activity of eNOS, the cells were pretreated with L-NAME (100 μM) for 30 min. *p $<$ 0.05 versus the mock control; #p $<$ 0.05 versus the Ang II treatment; †p $<$ 0.05 versus the tanshinone IIA and Ang II treatment. Notes: Cont, untransfected control; M, mock control; seNOS, eNOS siRNA transfection. (A) Effect of L-NAME, and eNOS siRNA on tanshinone IIA-decreased Ang II-induced ET-1 mRNA in cardiac fibroblasts. Cells were pretreated with or without tanshinone IIA (3 μM) for 30 min, and then treated with Ang II (100 nM) for 24 h. Results were shown as mean $\pm$ S.E.M. ($n = 4$). (B) Effect of L-NAME, and eNOS siRNA on tanshinone IIA-decreased Ang II-induced ET-1 secretion in cardiac fibroblasts. Cells were pretreated with or without tanshinone IIA (3 μM) for 30 min, and then treated with Ang II (100 nM) for 24 h. Results were shown as mean $\pm$ S.E.M. ($n = 5$). (C) Effect of L-NAME, and eNOS siRNA on tanshinone IIA-decreased Ang II-induced DNA synthesis in cardiac fibroblasts. Cells were pretreated with or without tanshinone IIA (3 μM) for 30 min, and then treated with Ang II (100 nM) for 24 h. Results were shown as mean $\pm$ S.E.M. ($n = 6$). (D) Effect of L-NAME, and eNOS siRNA on tanshinone IIA-decreased Ang II-induced cell proliferation in cardiac fibroblasts. Cells were pretreated with or without tanshinone IIA (3 μM) for 30 min, and then treated with Ang II (100 nM) for 24 h. Results were shown as mean $\pm$ S.E.M. ($n = 6$).

fibroblast proliferation induced by Ang II (data not shown). Our results demonstrate that tanshinone IIA inhibited Ang II-induced ET-1 expression and cell proliferation in cardiac fibroblasts. It is considered that ROS are mediators for intracellular signaling, which may involve the induction and/or the development of various physiological and pathophysiological events such as proliferation of cardiac fibroblasts (Cheng *et al.*, 2003). In addition,

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elevated ROS levels are involved in cell proliferation and ET-1 induction which can be attenuated by antioxidant pretreatment of cells (Cheng *et al.*, 2003). More specifically, tanshinone IIA also prevented Ang II-increase ROS generation (Fig. 3A). In particular, the suppression of ROS generation inhibits ET-1 expression, including our previous report, and has demonstrated that the activation of ERK is redox-sensitive, which may have the benefit effecting cell-protection (Cheng *et al.*, 2003). To gain insight into the mechanism of action of tanshinone IIA, we examined whether tanshinone IIA affected Ang II-activated ERK pathway of cardiac fibroblasts. As shown in Fig. 3B, exposure of cardiac fibroblasts to Ang II (100 nM) for 30 min rapidly activated phosphorylation of ERK. However, cardiac fibroblasts pretreated with tanshinone IIA (3 μM, 30 min) had decreased levels of Ang II-induced ERK phosphorylation significantly. Studies on the anti-oxidative effect of tanshinone IIA in inhibiting radical production revealed that it possesses a certain degree of protective effects *in vitro* and *in vivo*; including protection against injuries caused by ischemia/re-oxygenation (Fu *et al.*, 2007; Wu *et al.*, 1993; Yang *et al.*, 2008). The protective characteristics may be associated with their antioxidant activity.

It has been reported that NO can trigger apoptosis or protect cells from apoptotic stimuli, depending on the concentration of NO (Thomas *et al.*, 2008). In addition, NO has also been suggested to inhibit fibroblasts proliferation (Abdelaziz *et al.*, 2001; Calderone *et al.*, 1998). Tanshinone IIA can stimulate NO release from cultured human umbilical vein endothelial cells (Huang *et al.*, 2007; Lin *et al.*, 2006; Wu *et al.*, 2009). In this study, we demonstrated that tanshinone IIA treatment in cardiac fibroblasts significantly enhanced phospho-eNOS. Our findings reveal that tanshinone IIA increased NO production and the eNOS-NO signaling pathway was involved in tanshinone IIA's effect on Ang II-induced cardiac fibroblast proliferation.

In summary, this study has shown that tanshinone IIA inhibited Ang II-induced ROS formation, ERK phosphorylation, ET-1 expression, and cell proliferation. Moreover, tanshinone IIA also increased eNOS phosphorylation, and thereafter NO production in cardiac fibroblasts. It appears plausible that the Ang II-activated signaling pathway consists of a number of redox- sensitive steps and that tanshinone IIA treatment could modulate the redox state of the cell. The present study delivers important new insight into the molecular mechanisms of action of tanshinone IIA in cardiac fibroblasts.

Acknowledgments

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