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Research Paper

Hypertension-induced cardiac impairment is reversed by the inhibition of endoplasmic reticulum stress

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Keywords
endoplasmic reticulum stress; heart; hypertension; tauroursodeoxycholic acid

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Abstract

Objectives Endoplasmic reticulum stress (ERS) has been shown to play a crucial role in the pathogenesis of hypertension. However, the role and mechanisms of ERS on hypertension-induced cardiac functional and morphological changes remain unclear. In this study, the effect of ERS inhibition with tauroursodeoxycholic acid (TUDCA) on hypertension-induced cardiac remodelling was examined.

Methods Hypertension was induced by deoxycorticosterone-acetate (DOCA) and salt administration in uni-nephrectomized rats for 12 weeks. TUDCA was administered for the last four weeks. Rhythmic activity and contractions of the right atrium and left papillary muscle (LPM) were recorded. In the left ventricle, the expression of various proteins was examined and histopathological evaluation was performed.

Key findings Hypertension-induced increments in systolic blood pressure and ventricular contractions were reversed by TUDCA. In the hypertensive heart, while expressions of glucose-regulated protein-78 (GRP78), phospho-dsRNA-activated protein kinase-like ER kinase (p-PERK), sarcoplasmic reticulum Ca-ATPase-2 (SERCA2), matrix metalloproteinase-2 (MMP-2) and nuclear NF-κB p65 increased; Bcl-2 (B-cell lymphoma-2) expression decreased and the altered levels of all these markers were restored by TUDCA. In the microscopic examination, TUDCA treatment attenuated hypertension-stimulated cardiac inflammation and fibrosis.

Conclusions These results suggest that ERS inhibition may ameliorate cardiac contractility through improving ERS-associated calcium mishandling, apoptosis, inflammation and fibrosis, thereby offering therapeutic potential in hypertension-induced cardiac dysfunction.

Introduction

Hypertension is the most common chronic pathological condition characterized by systemic and persistent elevation of arterial blood pressure. Health problems related to hypertension account for approximately 13% of all deaths worldwide.[1] However, the underlying molecular mechanisms of hypertension and its associated complications are not well understood.

It is believed that prolonged, uncontrolled hypertension leads to cardiovascular diseases and some organ damage such as cardiac remodelling[2] which is one of the primary causes of morbidity and mortality in hypertension. Dysregulation of the intracellular Ca^{2+} cycle is important in the pathogenesis of cardiac remodelling and in the reduction of myocyte contractile function.[3] It has been shown that abnormal expressions of endoplasmic reticulum (ER) calcium-handling proteins, including SERCA2a and ryanodine receptor-2 (RyR-2) may contribute to impaired Ca^{2+} cycling and dysfunctions in the heart.[4,5] The ER is responsible for several essential cellular functions including synthesis and folding of proteins and calcium homoeostasis. Deterioration of any of the primary functions of ER leads to ERS via the activation of several cytoplasmic and nuclear signalling pathways, defined as the unfolded protein response (UPR).[6,7] UPR activation leads to increment of the expression of ER chaperones (such as GRP78), suppression of protein synthesis and activation of protein

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degradation to alleviate the burden on the ER.[7,8] This disruption in ER function results in impairment of excitation–contraction coupling followed by triggering of the inflammation, hypertrophy, fibrosis and apoptosis.[9,10]

Previous studies have indicated that ERS may play a pivotal role in the development of cardiovascular diseases including hypertension, ischaemic heart disease, atherosclerosis, cardiac hypertrophy and failure.[10–12] Pharmacological agents that alleviate ERS such as chemical chaperones have been reported to be beneficial in cardiovascular diseases.[13–16] The chemical chaperone TUDCA is produced endogenously in the liver and used for the treatment of primary biliary cirrhosis and ulcerative colitis.[17] It has been demonstrated that suppression of ERS with TUDCA has beneficial effects on cardiovascular complications in hypertensive and diabetic animals.[14–16] However, the role and significance of ERS in hypertension-induced cardiac remodelling have not been completely known yet. Therefore, in the present study, the effect of ERS inhibition by TUDCA treatment on functions of isolated cardiac tissues, several cardiac protein expressions related to ERS, inflammation, mitosis and apoptosis, and some cardiac histopathological alterations were investigated in the heart of DOCA-salt hypertensive rats.

Material and Methods

Chemicals

TUDCA was bought from Calbiochem (USA). DOCA, Noradrenaline, Ryanodine and all other agents were purchased from Sigma Chemical Co (USA). Antibodies were obtained from Milipore, Abcam, Santa Cruz and Cell Signalling. TUDCA was dissolved freshly in PBS. DOCA was prepared by dissolving in corn oil.

Animals and hypertension model

All experiments were performed considering Guide for the Care and Use of Laboratory Animals published by the National Institute of Health and approved by the Animal Ethics Committees of Gazi University (G.Ü.ET-13.076). Wistar rats (8-week-old male) were housed at 24 ± 1 °C with 12 h light–dark cycle. Animals were provided with food and drinking water ad libitum.

Hypertension was induced by modifying the DOCA-salt model used in the previous study of the current authors.[18] Animals were anaesthetized with intraperitoneal injection of ketamine + xylazine (60 mg/kg, 10 mg/kg respectively) and then left unilateral nephrectomy was performed in hypertensive groups. After a 7-day recovery period, the uni-nephrectomized rats were administered subcutaneously DOCA (20 mg/kg, two times a week) and throughout the 12-week experimental period, 1% NaCl + 0.2% KCl was supplemented to the drinking water.

The rats were randomly divided into four groups: group 1-control rats; group 2-DOCA-salt hypertensive rats; group 3-control rats treated with TUDCA for the last 4 weeks (150 mg/kg, i.p.); group 4-DOCA-salt hypertensive rats treated with TUDCA for the last 4 weeks (150 mg/kg, i.p.).

Systolic blood pressure was measured weekly using tail-cuff plethysmography (NIBP200A, COMMAT, Turkey). After the 12-week experimental period, all the animals were anaesthetized and blood samples were collected for biochemical measurements. The right atrium and LPM were rapidly excised, and ventricular tissue was collected for histology and protein expression analysis.

Isolated organ bath studies

To examine the role of the TUDCA treatment on the cardiac functions in resting and stimulated conditions, the experimental design described by Derici *et al.* was followed.[19] The isolated right atrium and LPM were mounted in isolated organ baths including aerated (5% CO_2 – 95% O_2) and heated (37°C) Krebs solution (112 mM NaCl, 5 mM KCl, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11.5 mM dextrose, 0.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, pH: 7.4). Isolated tissues were mounted between steel hooks and connected to the force-displacement transducer. Then, 2g initial tension was applied to tissues. LPMs were excited with 2 gold electrodes using a Harvard Apparatus Advanced Stimulator (Stimulation frequency 1 Hz, impulse duration 1 ms, voltage intensity 20% greater than the recordable contraction threshold). The isometric contractions and sinus rate were measured using force-displacement transducers (Grass FT.03) and recorded with a polygraph (Grass polygraph, model 79 D). All tissues were allowed to equilibrate for 40 min and washed at 10 min intervals with Krebs solution.

To examine the functional capacity of the SR calcium store in the noradrenaline-stimulated activities, the previously reported method was used.[20] After the equilibration duration, the Krebs solution was replaced with Ca^{2+}-free Krebs solution (including 10^{-5} M EGTA) for 15 min in order to remove extracellular Ca^{2+} from the tissues and then incubated with noradrenaline (10^{-6} M) for 5 min. Calcium was added incrementally to the bath solutions until last concentration of 2.5 mM was reached after noradrenaline incubation. The isolated cardiac tissues were washed and equilibrated for 40 min and then concentration–response curves of ryanodine (10^{-10} – 10^{-6} M) were performed by adding drug in the organ bath cumulatively. The findings were presented as a percentage of the baseline contraction or sinus rate. The responses of the right atrium were evaluated with the

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three parameters of developed tension (DT), resting tension (RT) and sinus rate.

Tissue homogenization and nuclear and cytoplasmic protein extraction

Homogenization and protein extraction (cytosolic/nuclear) were performed as described previously[21] with small modifications. Shortly, left ventricle samples were homogenized in 400–500 µl of STM buffer (including in mM; 250 sucrose, 50 Tris–HCl pH 7.4, 5 $MgCl_2$, protease and phosphatase inhibitor cocktails). The homogenate was incubated for 30 min on ice, mixed and then centrifuged at 700–800*g* for 15 min (4°C). The pellet was held on ice, and the supernatant was collected as cytosolic fraction.

The pellet (comprising nuclei + debris) was resuspended with 400–500 µl STM buffer, mixed and centrifuged several times. The final nuclear pellet was resuspended in 200–500 µl NET buffer (including: 20 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 500 mM NaCl, 1% Triton-X-100, 20% glycerol, protease and phosphatase inhibitors) until it became homogeneous. The homogenate was vortexed for 15–20 s and kept on ice for 30 min. The nuclei in this fraction were lysed with 10–15 passages through an 18-gauge needle on ice. The lysate was centrifuged at 10 000*g* for 30 min (4°C), and the final supernatant was labelled as nuclear fraction. The protein concentrations of supernatants were measured using the Lowry method.[22]

Western blot experiments

Equal amounts of protein extracts (50 – 100 µg) were separated by SDS-PAGE (10–12% acrylamide gel). The protein was transferred to PVDF membranes. After the transfer, the membranes were washed and were blocked for 1–1.5 h at room temperature with 4–5% non-fat dried milk or 3% BSA (for phospho proteins). Following blocking, the membranes were incubated with primary antibody: GRP78, PERK, p-PERK, Bax, Bcl-2, IκB-α, NF-κB p65, MMP-2, RyR-2, SERCA2, EGFR, ERK1/2, Akt, p-ERK1/2 and p-AKT overnight at 4 °C with gentle shaking.

After washing, horseradish peroxidase-conjugated secondary antibody was incubated for 1 h and the membranes were then incubated in Clarity™ Western ECL (Bio-Rad) solution. Images of the membranes were taken with the chemiluminescence detection system (Bio-Rad) connected with a CCD camera. GAPDH and Histone levels were used as loading controls for cytoplasmic and nuclear protein expression respectively. The results were assessed using the ImageLab 4.1 software and expressed as arbitrary units.

Histopathological examination of left ventricle

The left ventricle tissues isolated from all rats were washed rapidly with saline solution and then fixed in 10% buffered neutral formalin solution. All tissues were embedded in paraffin and then sectioned after fixation period. To evaluate cardiac hypertrophy and cardiac inflammation, the slices were stained with haematoxylin and eosin and then examined under a light microscope. Left ventricle sections were stained with Masson's Trichrome and microscopically evaluated for fibrosis.

The pathological hallmarks of cardiac hypertrophy were identified as myocyte hypertrophy and disarray together with expansion of the interstitial collagen compartment and these were evaluated as previously showed.[23] A 0–1 grading scale was used to evaluate the ventricular hypertrophy: 0: myocyte disarray < 20% or myocyte disarray 20% with pericellular fibrosis and 1: myocyte disarray 20% with patchy/extensive fibrosis or myocyte disarray > 20%. The interstitial fibrosis grading scale was used of 0: no change, 1: pericellular fibrosis, 2: patchy fibrosis and 3: extensive fibrosis. A 0–1 grading scale was used to quantify the perivascular fibrosis. The degree of left ventricular inflammation was evaluated as previously described.[24] The evaluation was performed on a scale ranging from grade 0 to 4: 0: no change, 1: low levels inflammatory cells infiltration, 2: moderate levels inflammatory cells and mild scarring, 3: high levels inflammatory cells infiltration and moderate scarring and 4: high levels inflammatory cells infiltration and heavy scarring.

Statistical analysis

All findings are given as mean ± SEM. Statistical analyses were carried out using the GraphPad Instat 5.01 (GraphPad Software, Inc., La Jolla, USA). The concentration–response curve to each agent was evaluated by repeated-measures of two-way ANOVA followed by the Bonferroni post-test. Statistical analyses of all other parameters were performed using the one-way ANOVA followed by the Dunnett post-test. $P < 0.05$ was considered at statistically significant.

Results

Systolic blood pressures of hypertensive DOCA-salt rats were markedly higher than the control rats (Control group 115.45 ± 1.92 mmHg; DOCA-salt group 202.68 ± 6.32 mmHg, $P < 0.05$) Treatment with TUDCA significantly decreased blood pressure in hypertensive rats (DOCA + TUDCA group 154.43 ± 5.57 mmHg; TUDCA group 103.82 ± 2.85, $P < 0.05$), as reported in our previous study.[25]

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To demonstrate the induction of ERS in the hypertensive heart, Western blotting was applied. The data indicated that the ERS markers, GRP78 and p-PERK expression, significantly increased in heart tissues from the DOCA-salt hypertensive rats ($P < 0.05$) but total-PERK expression did not change. The induction of ER stress markers in hypertensive myocardium was blunted in rats treated with TUDCA ($P < 0.05$) (Figure 1).

The effect of external calcium entry and intracellular release from SR on activities of isolated spontaneous beating right atrium and LPM was examined using calcium-free medium and external calcium administration. In the calcium-free medium, the basal (white column), noradrenaline (10^{-6} M)-stimulated (dotted column) and calcium-induced (stripped column) activities of the right atrium were similar in the control, DOCA and DOCA + TUDCA groups (Figure 2a,b and c). However, in the calcium-free medium, basal developed tension (white column), increased in the normotensive TUDCA group (Figure 2a). Noradrenaline (10^{-6} M)-stimulated contractions (dotted column) of LPM in the calcium-free medium were significantly higher in the hypertensive group and TUDCA treatment reversed the increased contraction ($P < 0.05$) (Figure 2d). Basal (white column) and noradrenaline (10^{-6} M)-stimulated contractions (dotted column) of LPM in the calcium-free medium were markedly lower in the normotensive TUDCA-treated animals ($P < 0.05$) (Figure 2d).

Ryanodine-induced negative inotropic responses of the right atrium were not different in all the groups (Figure 3a and 3b). The sinus rate of the right atrium in higher ryanodine concentration (10^{-6} M) was significantly lower in the hypertensive DOCA group ($P < 0.05$) (Figure 3c). Ryanodine (10^{-6} M)-induced negative inotropic responses of LPM were markedly higher in the hypertensive group ($P < 0.05$) (Figure 3d). TUDCA treatment did not affect these changes (Figure 3).

It is known that ryanodine receptor-2 is the predominant form of ryanodine receptor in heart, and it plays an important role in cardiac dysfunction. Therefore, ryanodine receptor-2 expression was examined in the left ventricle. Consistent with results from isolated tissue bath, ryanodine receptor-2 expression in the left ventricle was similar in all the groups (Figure 4a and 4c). SERCA2 expression

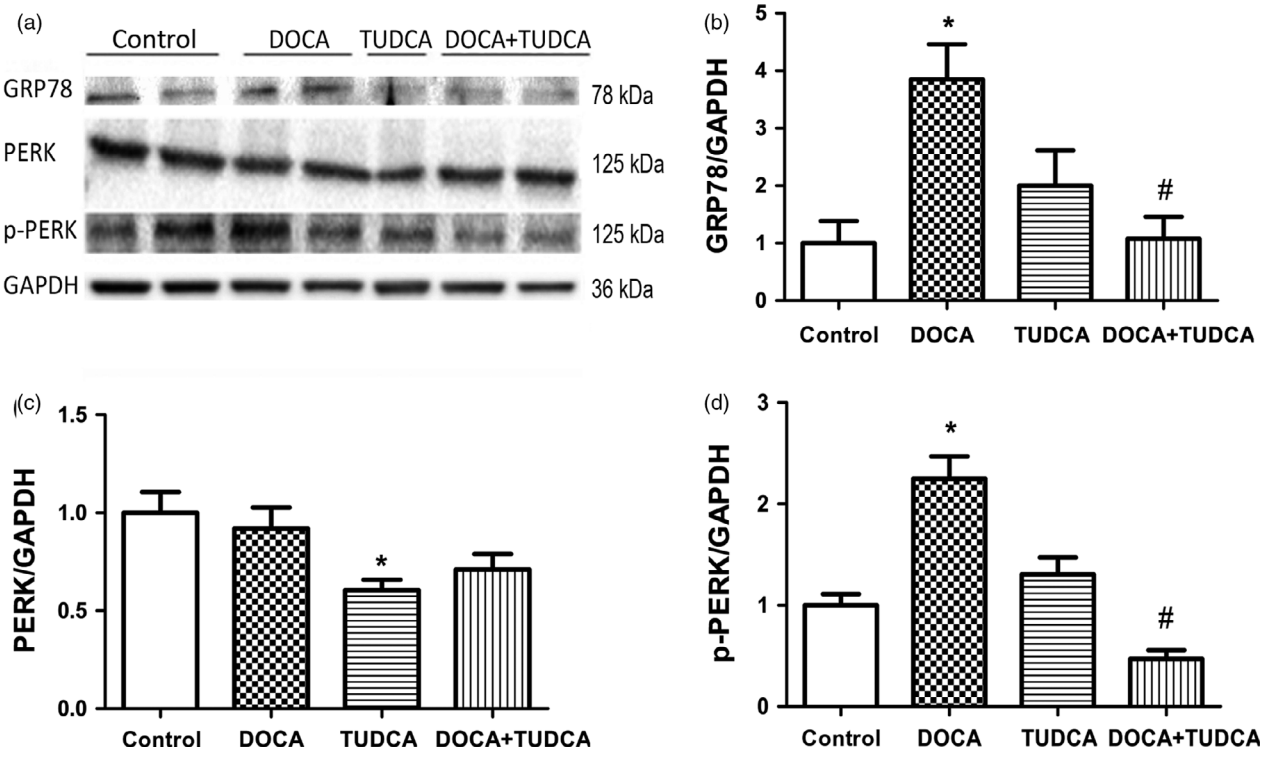


Figure 1 The effects of TUDCA treatment on expression of ERS markers in the left ventricular tissue. Representative Western Blot images for GRP78, total-PERK and phospo-PERK (a). Densitometric analysis of blots for GRP78 (b), total-PERK (c) and phospo-PERK (d). TUDCA treatment markedly inhibited cardiac endoplasmic reticulum stress induced by DOCA-salt hypertension. * indicates the significant difference from Control group ($P < 0.05$); # indicates the significant difference from DOCA-salt hypertensive group ($P < 0.05$). Results are given as mean ± SEM. (*n*: 4–8).

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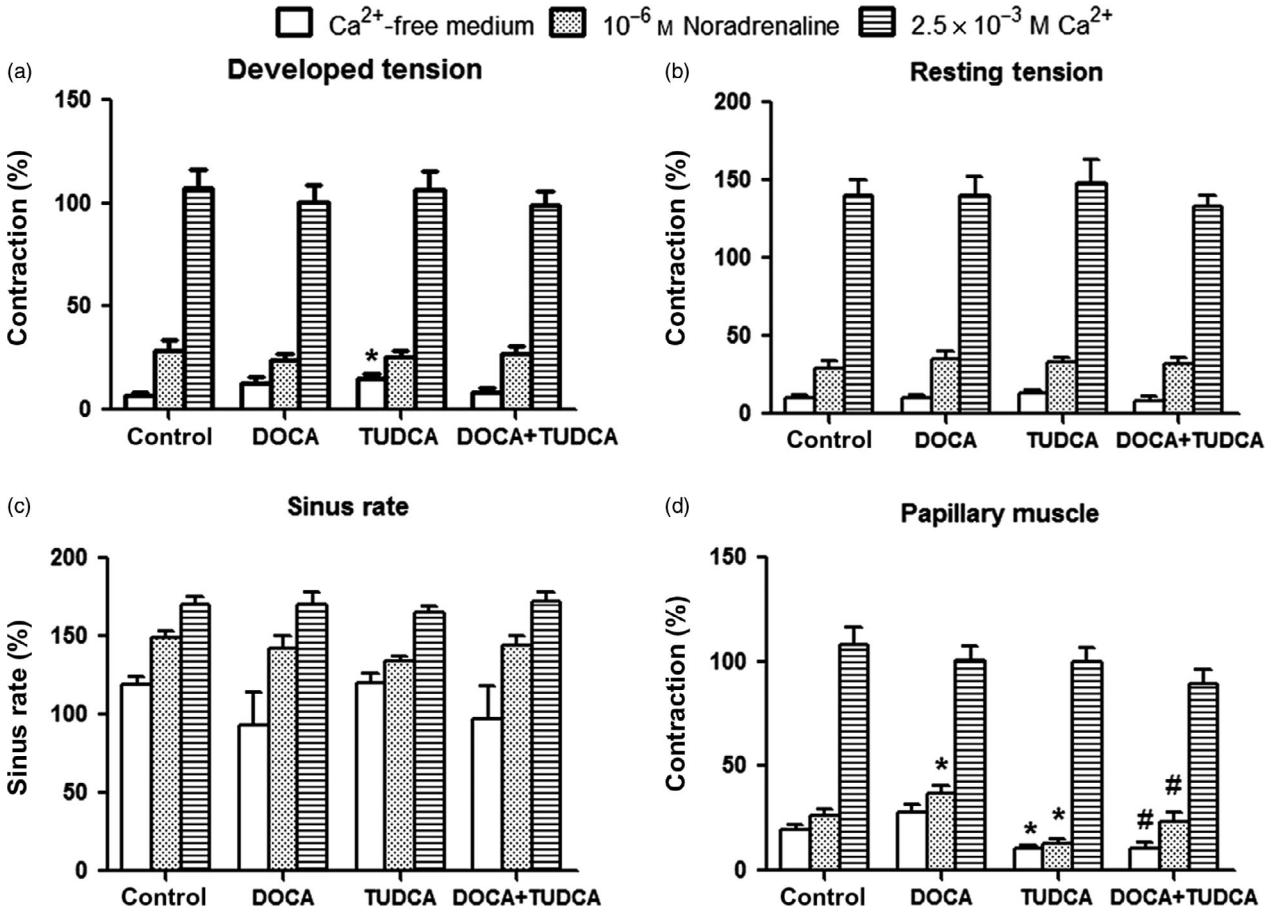


Figure 2 The effect of TUDCA treatment on rhythmic activity and contractions of the right atrium and the LPM isolated from rat hearts. Developed tension (a), resting tension (b) and sinus rate (c) of the right atrium and contraction of the LPM (d). Noradrenaline (10^{-6} M)-induced contraction of the LPM in Ca^{2+}-free medium significantly increased in hypertensive groups, TUDCA treatment significantly reversed basal and noradrenaline (10^{-6} M)-mediated contractions. * indicates the significant difference from Control group ($P < 0.05$); # indicates the significant difference from DOCA-salt hypertensive group ($P < 0.05$). Results are given as mean ± SEM (n: 6–12).

significantly increased in the hypertensive group but TUDCA treatment attenuated this protein expression in the left ventricle ($P < 0.05$) (Figure 4b and 4d).

While the proapoptotic marker Bax expression was similar in all groups, antiapoptotic marker Bcl-2 expression levels decreased in the hypertensive heart ($P < 0.05$) (Figure 5a,b and c). TUDCA treatment significantly enhanced Bcl-2 expression in the hypertensive group ($P < 0.05$) (Figure 5a and 5c). In addition, profibrotic marker MMP-2 protein expression markedly augmented in the hypertensive group ($P < 0.05$) and it was slightly decreased by TUDCA treatment (Figure 5a and 5d).

For determining the mechanisms underlying the cardioprotective actions of ERS inhibition, the effects of TUDCA on cardiac inflammation were investigated in DOCA-salt hypertensive rats. The expression of IκB-α, a cytoplasmic inhibitor of NF-κB, was not different between the groups (Figure 6a and 6b). The NF-κB p65 protein expression markedly increased in the nuclear fraction ($P < 0.05$) (Figure 6d) but was unchanged in the cytosolic fraction of hypertensive heart tissue (Figure 6c). TUDCA treatment significantly decreased nuclear NF-κB p65 expression in the hypertensive group ($P < 0.05$) (Figure 6d).

Cardiac inflammation was evaluated using haematoxylin and eosin staining. As shown in Table 1 and Figure 7, inflammatory cell infiltration in the left ventricle was markedly higher in DOCA-salt rats compared with the control group ($P < 0.05$) and these cells were generally located in scar sites and the areas of fibrosis. TUDCA treatment slightly decreased inflammatory cells infiltration throughout the left ventricle ($P > 0.05$). The excess inflammatory cell accumulation was consistent with the increased collagen synthesis and deposition in the left ventricle. In both interstitial and perivascular sites of the left ventricle, collagen content was higher in hypertensive rats compared with control rats ($P < 0.05$). TUDCA treatment significantly

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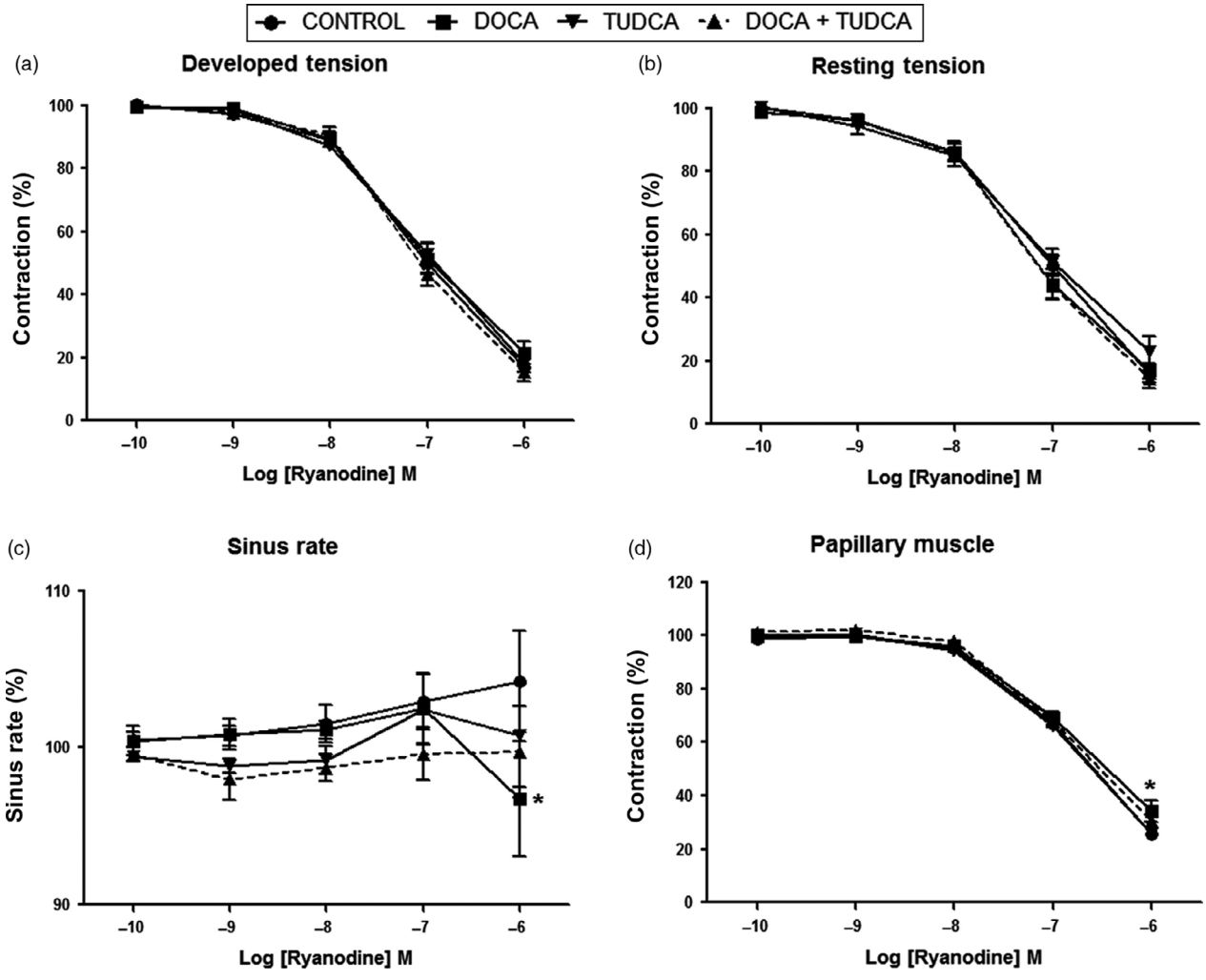


Figure 3 The effect of TUDCA treatment on the ryanodine responses in the right atrium and LPM isolated from rat heart. Developed tension (a), resting tension (b), sinus rate (c), LPM contraction (d). High ryanodine concentration-induced negative inotropic responses of the LPM were markedly greater in the hypertensive group. TUDCA administration did not affect the ryanodine-induced responses of tissues. * indicates the significant difference from Control group ($P < 0.05$). Results are given as mean ± SEM (n: 6–12).

attenuated ($P < 0.05$) perivascular fibrosis but did not change the intensity of interstitial fibrosis in hypertensive animals (Table 1 and Figure 7).

The pathological characteristics of ventricular hypertrophy are myocyte hypertrophy and disarray with enlargement of the interstitial collagen compartment. While cardiac fibrosis was induced by the DOCA-salt hypertension, cardiac hypertrophy was not observed. TUDCA treatment did not affect the ventricular hypertrophy score (Table 1 and Figure 7).

Mitotic pathways also play a crucial role in the development of myocardial hypertrophy associated with cardiac remodelling. Therefore, the expressions of some molecules in the epidermal growth factor (EGF) signalling pathway were examined. In the left ventricle, expressions of EGF receptor (EGFR) and its downstream effectors Akt, ERK1/2, p-Akt and p-ERK1/2 (markers of cardiac hypertrophy) were similar in all groups (Figure 8).

Discussion

The findings of the present study demonstrated that ERS inhibition in hypertension might prevent cardiac functional and structural changes possibly through mechanisms associated with improvement of intracellular Ca^{2+} handling and suppression of some transcription factors.

It is well known that increased arterial blood pressure is associated with cardiac remodelling, such as disturbances of heart contractility and rhythm, cardiac inflammation, hypertrophy, fibrosis and apoptosis. It has recently been

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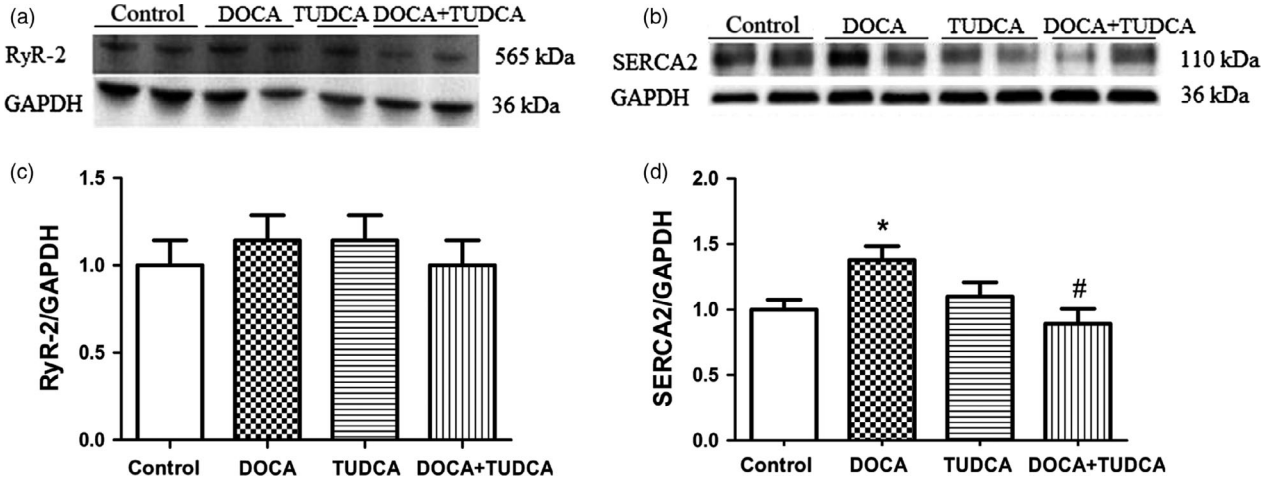


Figure 4 The effects of TUDCA treatment on expressions of RyR-2 and SERCA2 in the left ventricular tissue. Representative Western Blot images (a and c) and densitometric analysis of blots for RyR-2 (b) and SERCA2 (d). RyR-2 expression was similar in all groups. Cardiac SERCA2 expression significantly increased in the hypertensive group and TUDCA administration reversed this elevation. * indicates the significant difference from Control group ($P < 0.05$); # indicates the significant difference from DOCA-salt hypertensive group ($P < 0.05$). Results are given as mean ± SEM (n: 4–8).

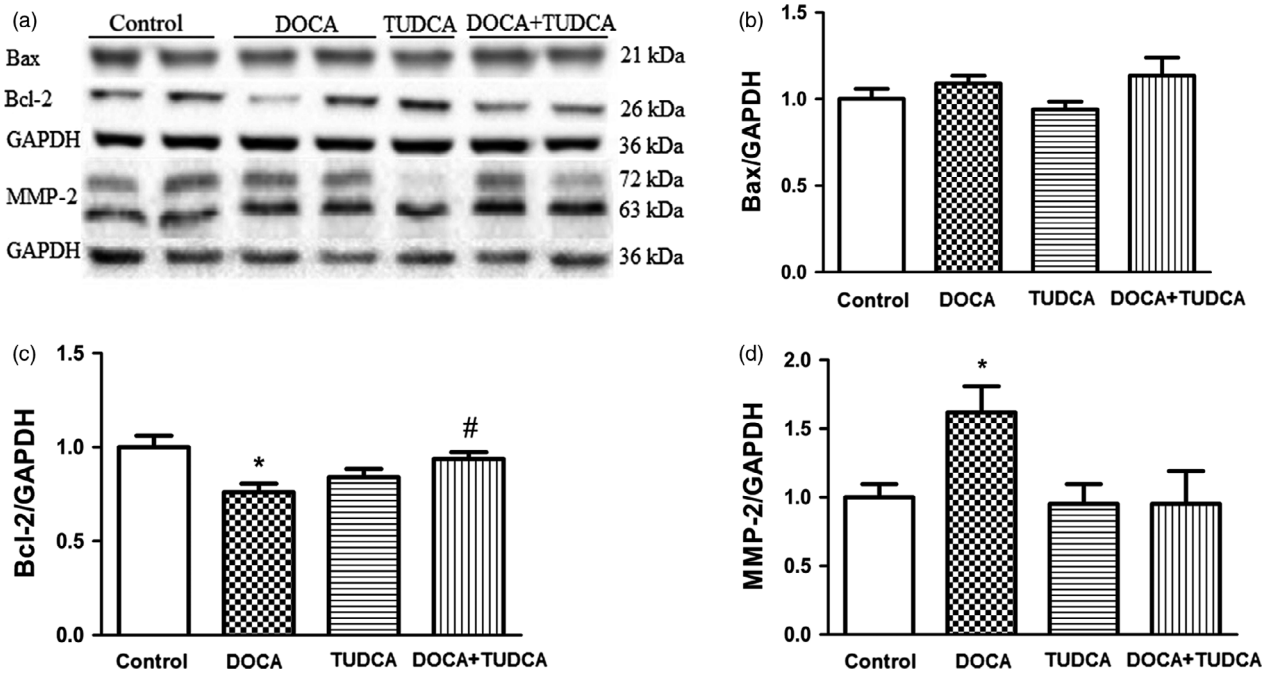


Figure 5 The effects of TUDCA treatment on expression of apoptotic and profibrotic markers in the left ventricular tissue. Representative Western Blot images for Bax, Bcl-2 and MMP2 (a). Densitometric analysis of blots for Bax (b), Bcl-2 (c) and MMP-2 (d). Proapoptotic marker Bax expression was similar in all groups. Antiapoptotic marker Bcl-2 expression decreased in hypertensive hearts but was reversed by TUDCA treatment. DOCA-salt hypertension increased cardiac MMP-2 expression but TUDCA slightly attenuated it. * indicates the significant difference from Control group ($P < 0.05$); # indicates the significant difference from DOCA-salt hypertensive group ($P < 0.05$). Results are given as mean ± SEM (n: 4–8).

reported that ERS plays an important role in several cardiovascular diseases[10–12] but the role and the significance of ERS in hypertension-induced cardiac functional and structural changes are not fully elucidated.

During the ERS, three ER transmembrane sensor proteins PERK, activating transcription factor 6 (ATF6) and inositol requiring kinase 1 (IRE1) are separated from chaperone protein GRP78 which plays a critical role in UPR.

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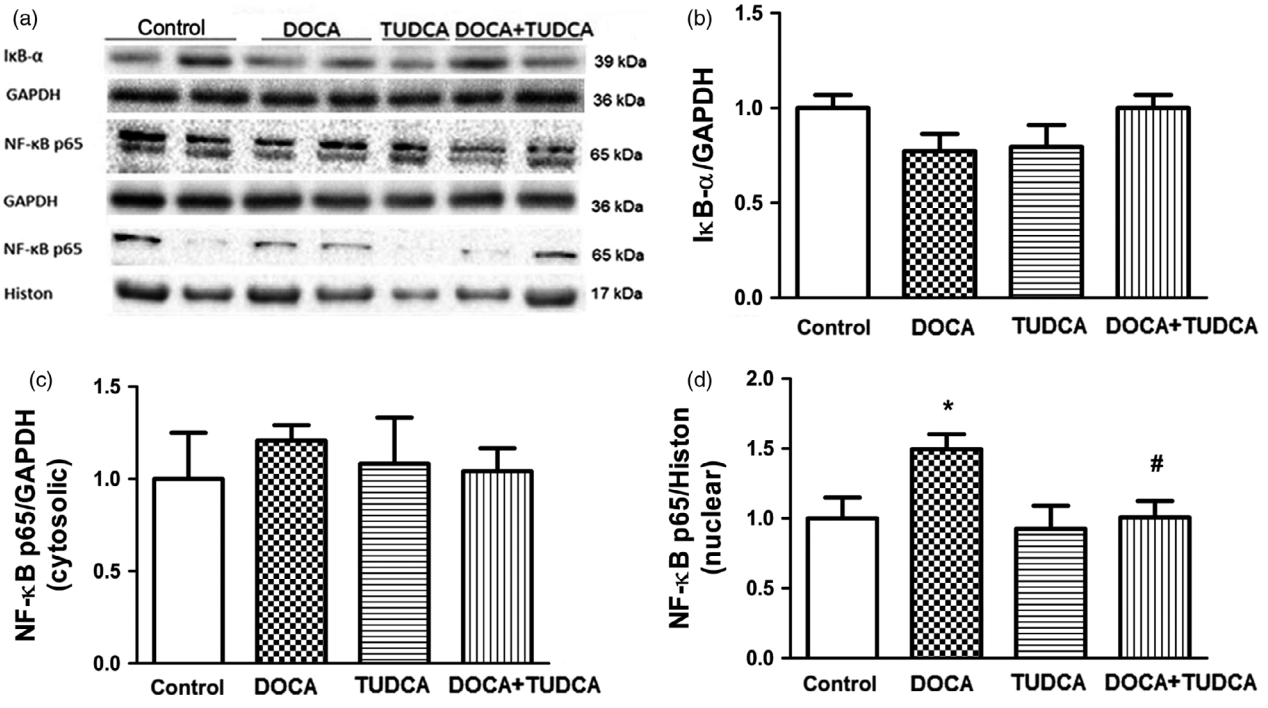


Figure 6 The effects of TUDCA treatment on the expression of inflammatory markers in the left ventricular tissue. Representative Western Blot images for IκB-α, cytosolic and nuclear NF-κB p65 subunit (a). Densitometric analysis of blots for IκB-α (b), cytosolic (c) and nuclear (d) NF-κB p65 subunit. Nuclear NF-κB p65 expression markedly augmented in the hypertensive group but it was significantly reduced by TUDCA administration. * indicates the significant difference from Control group ($P < 0.05$); # indicates the significant difference from DOCA-salt hypertensive group ($P < 0.05$). Results are given as mean ± SEM (n: 4–8).

Table 1 Effect of TUDCA treatment on histopathology of heart.

Parameters	CONTROL	DOCA-salt	TUDCA	DOCA + TUDCA
Ventricular hypertrophy score	0.35 ± 0.13	0.62 ± 0.18	0.20 ± 0.13	0.83 ± 0.16
Cardiac inflammation score	1.35 ± 0.17	2.25 ± 0.31*	0.90 ± 0.10	1.83 ± 0.17
Interstitial fibrosis score	1.07 ± 0.22	2.38 ± 0.26*	1.10 ± 0.10	2.17 ± 0.17
Perivascular fibrosis score	0.07 ± 0.07	0.88 ± 0.13*	0.10 ± 0.10	0.33 ± 0.21#

Values are expressed as mean ± SEM. $P < 0.05$, Differences from Control (*) and DOCA-salt (#).

PERK, activated by autophosphorylation triggers various signal transduction pathways to restore the ER homoeostasis.[7,8] It has been shown that cardiac GRP78 and p-PERK expression increase in pressure overload mice and spontaneously hypertensive rats.[26,27] In the current study, ERS inhibition with TUDCA treatment reduced the increment in GRP78 and p-PERK expression induced by hypertension. The present findings demonstrate that TUDCA treatment may attenuate hypertension-induced cardiac ERS and its related damages.

It has been reported that ERS inhibition with TUDCA reduces the blood pressure in many studies using different models of hypertension.[15,16,28] Parallel to these evidences, it has been demonstrated that TUDCA treatment significantly decreased systolic blood pressure of DOCA-salt hypertensive rats in the present study. These findings suggest that ERS is a critical factor in the elevation of blood pressure in the DOCA-salt hypertension model.

The abnormalities in intracellular Ca^{2+} cycling in cardiomyocytes may contribute to ERS induction in the heart.[29] However, functional reflection of intracellular calcium dysregulation has not yet been achieved. In this study, the effect of TUDCA treatment on the change in cardiac contractility and rhythm was examined in the heart of hypertensive rats induced by DOCA-salt treatment. In the calcium-free medium, noradrenaline-stimulated contraction of ventricular tissue was higher in the DOCA-salt hypertensive group than the control and TUDCA treatment reversed this increase. In addition, the highest concentration of ryanodine-induced decrease in contraction of the

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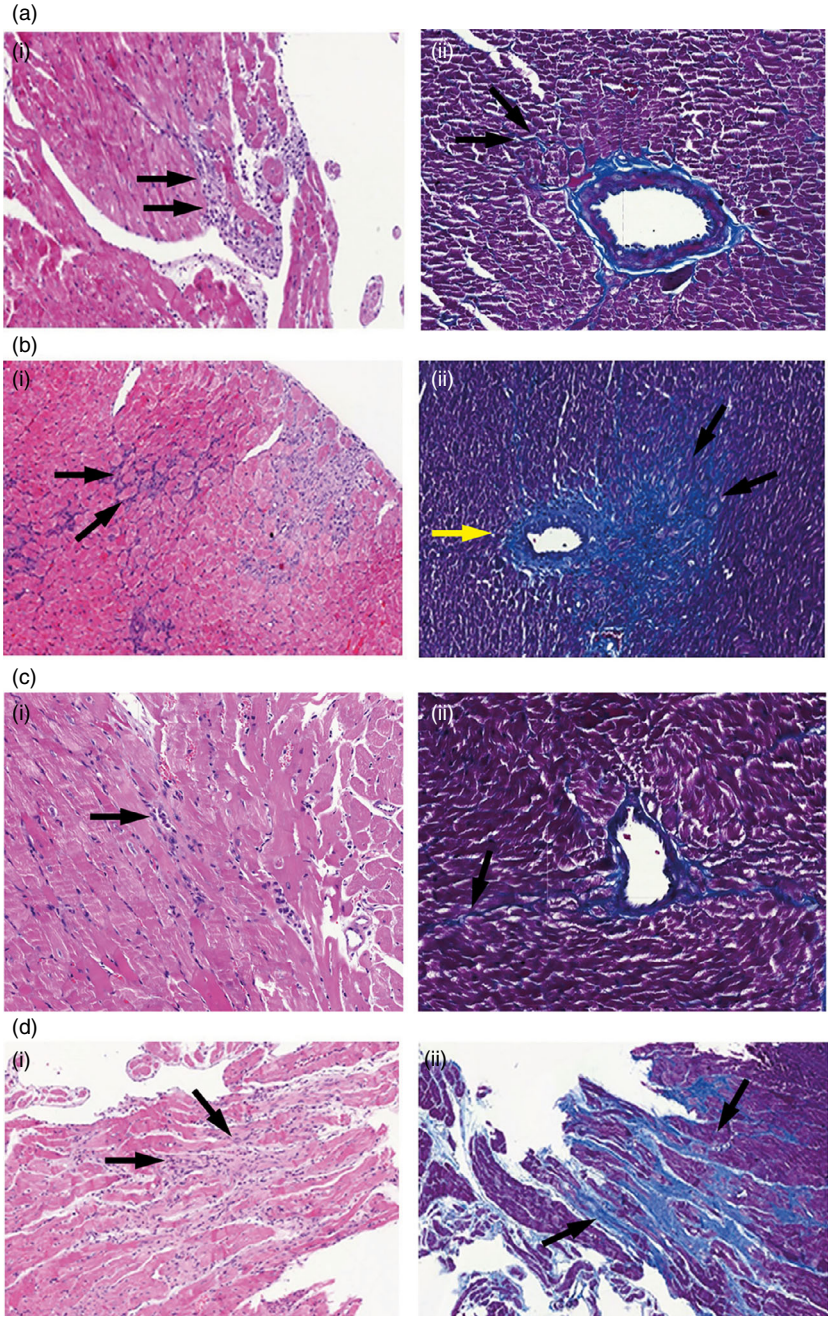


Figure 7 Microscopic examination of ventricular tissue. Haematoxylin and eosin staining (H&E) and Masson's Trichrome (MT) staining of left ventricular tissue (a, b, c, d). Figure 7a; Control group i. no ventricular hypertrophy [scale: 0], low inflammation (black arrow) [scale: 1], (H&E, ×40), ii. no perivascular fibrosis [scale: 0], pericellular fibrosis (black arrow) [scale: 1], (MT, ×40). Figure 7b; DOCA-salt group i. no ventricular hypertrophy [scale: 0], moderate inflammation (black arrow) [scale: 2], myocyte disarray (H&E, ×40) ii. perivascular fibrosis (yellow arrow) [scale: 1], patchy fibrosis (black arrow) [scale: 2], (MT, ×40). Figure 7c; TUDCA group i. no ventricular hypertrophy [scale: 0], low inflammation (black arrow) [scale: 1], (H&E, ×40) ii. no perivascular fibrosis [scale: 0], pericellular fibrosis (black arrow) [scale: 1], (MT, ×40). Figure 7d; DOCA + TUDCA group i. no ventricular hypertrophy [scale: 0], moderate inflammation (black arrow) [scale: 2], (H&E, ×40) ii. no perivascular fibrosis [scale: 0], patchy fibrosis (black arrow) [scale: 2], (MT, ×40). [Colour figure can be viewed at wileyonlinelibrary.com]

LPM was markedly less in the hypertensive group than in the control group, but ERS inhibition with TUDCA did not affect ryanodine responses in the left ventricle. These results suggest that ERS inhibition may ameliorate calcium dysregulation induced by hypertension in left ventricular tissue, and in particular, might regulate Ca^{2+} deposition in SR.

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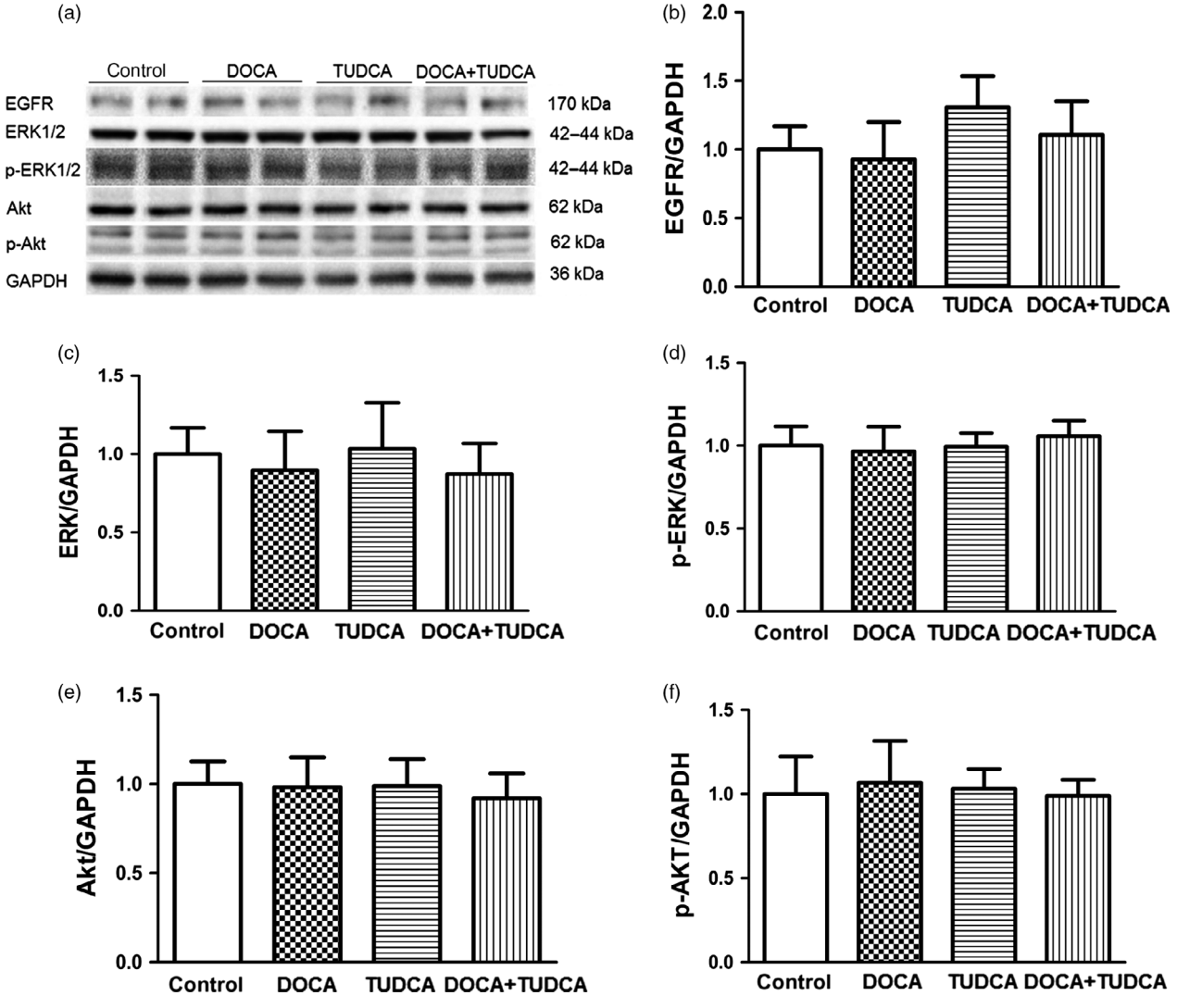


Figure 8 The effects of TUDCA treatment on the expression of EGFR and its downstream effectors. Representative Western Blot images for EGFR, ERK1/2, p-ERK1/2, Akt and p-Akt (a). Densitometric analysis of blots for EGFR (b), ERK1/2 (c), p-ERK1/2 (d), Akt (e) and p-Akt (f). Expressions of these proteins were similar in all groups. Results are given as mean ± SEM ($P > 0.05$) (n: 5–6).

It has been reported that abnormal expression of calcium-handling proteins, including SERCA2a and ryanodine receptor-2 may contribute to impaired Ca^{2+} cycling and contractile functions in the heart.[4,5] ER is a major Ca^{2+} store and disturbances in Ca^{2+} homoeostasis of cardiac myocytes may be the result of ERS. In the present study, SERCA2 expression significantly increased in the hypertensive group and TUDCA treatment attenuated this protein expression in ventricular tissue, but ryanodine receptor-2 expressions were similar in all the groups. This result can be explained by noradrenaline-stimulated contractions of LPM in the calcium-free medium. This study is the first to show that ERS inhibition ameliorated Ca^{2+} deposition via SERCA2 in the hypertensive heart. These results suggest that ER Ca^{2+} homoeostasis may be disrupted possibly due to alterations of ER Ca^{2+} deposition in the hypertensive heart and TUDCA treatment may improve these changes and restore cardiac contractility.

The loss of cardiomyocytes induced by apoptosis is a crucial mechanism contributing to cardiac remodelling in response to hemodynamic overload.[30,31] It has been shown that prolonged or excessive ERS may induce apoptosis in several pathologies.[29] In this study, it was shown that the antiapoptotic marker, Bcl-2 expression, was significantly reduced but the proapoptotic marker, Bax expression, did not change in hypertensive hearts. In addition, the fibrotic marker, MMP-2 protein expression, in the ventricle significantly increased in the hypertensive group. TUDCA

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treatment increased Bcl-2 expression and slightly decreased MMP-2 expression without altering Bax expression in the heart. Consistent with these findings, Spitler *et al.* showed that aortic protein expression of Bcl-2 was reduced in the SHRs and was restored in SHR treated with TUDCA.[16] These results suggest that apoptosis and fibrosis, which indicate tissue damage, might be triggered by hypertension and ERS inhibition by TUDCA may alleviate the development of these pathologies in heart.

Previous studies have reported that chronic inflammation contributes to the development of hypertension-induced cardiac remodelling.[32–35] The transcription factor NF-κB regulates several genes involved in the activation of stress, cell proliferation and inflammation. Under normal conditions, inactive NF-κB dimers (generally p65/p50) are connected to inhibitor of κB (IκB) in the cytoplasm. During the stimulation, IκB is phosphorylated by IKB kinases and separated from NF-κB, leading to nuclear translocation of NF-κB.[36] Recent evidence indicates that ERS is implicated in inflammatory processes (specifically NF-κB pathway) and plays rather fundamental roles on the onset and progression of cardiovascular diseases.[37–39] However, its role has not been reported in hypertension-associated cardiac inflammation process. The findings of the current study showed that nuclear NF-κB p65 subunit expression increased but IκB-α expression did not change in hypertensive hearts. Nuclear NF-κB p65 subunit expression was reduced after ERS inhibition with TUDCA. These results are consistent with those of previous studies[39,40] and demonstrate for the first time the importance of ERS in the regulation of inflammation in hypertensive heart. It was also observed that inflammatory cell infiltration markedly increased in the hearts of hypertensive rats as indicated by microscopic examination. TUDCA treatment slightly decreased inflammatory cell infiltration in heart tissue. These findings suggest that hypertension can lead to inflammation in the heart and ERS inhibition might attenuate cardiac inflammation.

Previous studies have shown that cardiac hypertrophy and fibrosis develop in hypertensive animals and patients.[41,42] Cardiac hypertrophy is a progressive event characterized by alterations in cardiomyocyte size in hypertension.[43] Recently, mitotic pathways, such as EGFR signalling, have been suggested to be associated with the development of hypertension and cardiac hypertrophy.[44] Transactivation of EGFR, which initiates both the activation of ERK1/2, PI3K and Akt (protein kinase B), mediates the hypertrophic response.[45,46] In the current study, the ventricular hypertrophy score was similar in all groups. In accordance with these results, EGFR and its downstream effectors such as Akt, ERK1/2, p-Akt and p-ERK1/2 expressions in the heart did not show any difference between all groups. However, cardiac fibrosis was significantly induced in the hypertensive heart. ERS inhibition with TUDCA blunted perivascular fibrosis and slightly decreased the intensity of interstitial fibrosis in the heart of hypertensive animals. In parallel to these results, Rani *et al.* demonstrated that TUDCA attenuates pressure overload-induced cardiac hypertrophy and fibrosis.[47] Similar to previous findings, the results of the current study indicated that inhibition of ERS could be a beneficial therapeutic strategy to reverse cardiac morphological changes.

Conclusion

In conclusion, our findings have demonstrated that TUDCA protects against alterations of cardiac contractility and rhythm, intracellular Ca^{2+} mishandling, cardiac inflammation, hypertrophy, fibrosis and apoptosis in DOCA-salt hypertension. The cardioprotective effects of the ER chaperone might be associated with improved intracellular Ca^{2+} signalling and alleviated inflammatory pathways. The results of the present study suggest that ERS inhibition could be a novel therapeutic strategy to reverse hypertension-induced cardiac pathologies.

Declarations

Conflict of interest

The Author(s) declare (s) that they have no conflicts of interest to disclose.

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