

# Enalapril attenuates endoplasmic reticulum stress and mitochondrial injury induced by myocardial infarction via activation of the TAK1/NFAT pathway in mice

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**Abstract.** The present study investigated the effect of enalapril on myocardial infarction (MI) and its mechanism of action in mice. Treatment with enalapril significantly attenuated cellular apoptosis and death. *In vivo*, enalapril treatment alleviated MI injury, and decreased myocardial apoptosis and the size of the infarct area. This was paralleled by increased Bcl-2 expression, decreased Bax expression, a decreased caspase-3 level, decreased expression of endoplasmic reticulum stress-associated proteins, including activating transcription factor 6 and 78 kDa glucose-regulated protein, and fewer TUNEL-positive cells in the heart. Furthermore, enalapril-treatment increased transforming growth factor-activated kinase 1/nuclear factor of activated T cells 3 signaling, which protected the myocardium.

## Introduction

Although there are numerous treatment methods for ischemic coronary heart disease, this condition remains the leading cause of mortality worldwide and is often associated with sudden death (1). Apoptosis, a well-defined process, serves an important role in myocardial infarction (MI), via both the extrinsic and intrinsic pathways (2,3). Several studies have indicated that the renin-angiotensin system (RAS) is activated following MI and the release of angiotensin II (Ang II) leads to myocardial damage (4).

Enalapril, an angiotensin-converting enzyme inhibitor, significantly decreases MI-associated mortality (5-7). Over the previous 2 decades, there has been a marked increase in the use of enalapril-treatment, which has contributed to the decreased rates of mortality observed (8). Furthermore, treatment with enalapril significantly prevents apoptosis, endoplasmic reticulum (ER) stress and mitochondrial injury (9). However, to the best of our knowledge, the underlying mechanism of the effect of enalapril on MI remains uncertain.

Transforming growth factor-activated kinase 1 (TAK1) serves an important role as a survival signal. The deletion of TAK1 induces apoptosis in hematopoietic cells and hepatocytes, resulting in bone marrow and liver failure in mice, and the downregulated expression of pro-survival genes (10). In an additional study, TAK1 was demonstrated to drive cell survival as a responsive kinase (11). Furthermore, TAK1 has been suggested to inhibit apoptosis in numerous cell lines. For example, TAK1-silencing induced apoptosis of lymphoma cells (12). Another study confirmed increased apoptosis levels in TAK1-deficient B cells (13). Furthermore, TAK1 serves as a survival signal and protects hematopoietic stem and progenitor cells from apoptosis (14). Nuclear factor of activated T cells 3 (NFAT3) is regulated by calcium signaling. De Windt *et al* (15) suggested that NFAT3 is associated with the likely mechanism of calcineurin-induced protection against apoptosis in myocytes. In addition, inhibition of NFAT3 has been confirmed to induce apoptosis in cortical neurons (16). However, to the best of our knowledge, the role of the TAK1/NFAT3 pathway in MI treated with enalapril remains unknown. Therefore, the present study aimed to determine the mechanism of enalapril-treatment for MI in mice.

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**Abbreviations:** Ang II, angiotensin II; ERS, endoplasmic reticulum stress; MI, myocardial infarction; NFAT3, nuclear factor of activated T cells 3; TAK1, transforming growth factor- $\beta$ -activated kinase 1; RAS, rennin-angiotensin system

**Key words:** enalapril, myocardial infarction, cardiac remodeling, transforming growth factor- $\beta$ -activated kinase 1/nuclear factor of activated T cells pathway, apoptosis

## Materials and methods

**MI mouse model and enalapril treatment.** All animal experiments were approved by the Animal Ethics Committee of Wenzhou Medical University (no., wydw2017-0007) and were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (17). Adult male C57BL/6 mice (20–25 g) aged 6–8 weeks (Shanghai Laboratory Animal Center) were housed (temperature,  $24 \pm 1^\circ\text{C}$ ; humidity,  $45 \pm 10\%$ ) in a specific pathogen-free environment, with access to standard food and sterile tap water *ad libitum* under 12 h light/dark cycles and allowed to acclimatize for  $\geq 72$  h prior to surgery. The animal use and care protocol conformed to the Guide for the Care and Use of Laboratory Animals (17). All surgical procedures were performed under anesthesia using intraperitoneal (i.p.) injection of ketamine (90 mg/kg body weight) and xylazine (5 mg/kg body weight) (18). Anesthesia was assessed by measuring the toe pinch reflex. Adequate anesthesia resulted in a complete lack of response in the extremity. A left thoracotomy was performed via the fourth intercostal space, the heart was exposed and the pericardium was opened. The left anterior descending coronary artery (LAD) was ligated with a 7-0 silk suture near its origin between the pulmonary outflow tract and the edge of the left atrium. Acute myocardial ischemia was deemed successful when the anterior wall of the left ventricle (LV) became pale and when echocardiography demonstrated a decreased ejection fraction 1 week following surgery. Sham-operated mice were subjected to the same procedure, but the suture around the LAD was not tied. Animals were kept on a heating pad until they awoke. Mice that survived surgery were randomly assigned to different treatment groups ( $n=6-10$  per group). Animals that underwent the same surgical procedure without coronary artery ligation served as a control group ( $n=6-10$  per group). MI mice were treated with a dose of 20 mg/kg (19,20) enalapril (Sigma-Aldrich; Merck KGaA) or an equal amount of drinking water daily through gastric gavage, following echocardiography, for 3 weeks. These groups were termed MI + Ena and MI groups, respectively.

**Echocardiography.** At the end of treatment, cardiac systolic function was measured under anesthesia with thiopentone (method, intraperitoneal injection; dose, 20 mg/kg body weight) (21). Mice were kept on a heating pad in the left lateral decubitus or supine position and two-dimensional images were recorded. LV parameters, including the internal diastolic diameter (LVIDd) and internal systolic diameter (LVIDs), were obtained from M-mode interrogation in the long-axis view. The LV percentage fractional shortening (LV%FS) and LV ejection fraction (LVEF) were calculated as follows:  $\text{LV\%FS} = (\text{LVIDd} - \text{LVIDs}) / \text{LVIDd} \times 100$ ; and  $\text{LVEF} = [(\text{LVIDd})^3 - (\text{LVIDs})^3] / (\text{LVIDd})^3 \times 100$ . All echocardiographic measurements were averaged from at least 3 independent cardiac cycles.

**Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay.** At the end of the treatment period, all mice were euthanized with an overdose of sodium pentobarbital (200 mg/kg, i.p.). Animal death was verified by observation of cardiac arrest and pupil enlargement for

1 min. The humane endpoints established in this study were as follows: Impaired ambulation that prevented animals from reaching food or water; excessive weight loss and extreme emaciation; lack of physical or mental alertness; difficult labored breathing; and prolonged inability to remain upright. Animals were observed a minimum of twice daily, with more frequent observations immediately after dosing and when increased morbidity or mortality was expected (17). The heart was then removed and fixed in 10% formalin for 24–48 h at  $4^\circ\text{C}$ . Formalin-fixed heart tissues were then embedded in paraffin and sections were cut ( $\sim 4 \mu\text{m}$  thick). Cardiomyocyte apoptosis was detected using a one-step TUNEL Apoptosis assay kit at  $37^\circ\text{C}$  for 1 h (Roche Diagnostics GmbH), according to the manufacturer's protocol, followed by DAPI staining ( $10 \mu\text{g/ml}$ ; Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 2 min. Anti-fade mounting medium was then added (cat. no. P0126; Beyotime Institute of Biotechnology) to each slide. Images were obtained from five fields per slide using confocal microscopy (magnification  $\times 400$ ; NIKON A1R/A1; Nikon Corporation).

**Masson's trichrome (MT) staining and immunohistochemistry.** Heart tissues were processed as described above. Fibrosis was assessed by MT staining (Beijing Suolai Bao Technology Co., Ltd.), according to the manufacturer's protocol in addition to the protocol described previously (22). Briefly, the tissue sections were stained with ponceau for 7 min, aniline blue for 7 min and phosphomolybdic acid for 2 min at room temperature.

Immunohistochemistry (confocal, magnification  $\times 400$ ) was performed to detect associated proteins, including c-caspase 3 and NFAT3. Briefly, the slides were heated at  $60^\circ\text{C}$  for 1 h, deparaffinized and hydrated with xylene, graded ethyl alcohols and  $\text{dH}_2\text{O}$ . Following antigen retrieval [7 min of boiling and 3 min in sodium citrate buffer (10 mM, pH 6.0) using an induction cooker], the sections were treated for 15 min with 3%  $\text{H}_2\text{O}_2$  and blocked for 30 min with 10% goat serum (Abcam) at room temperature. The sections were then incubated overnight at  $4^\circ\text{C}$  with anti-cleaved caspase-3 (cat. no. 9694; Cell Signaling Technology, Inc.; 1:1,000) and anti-NFAT3 (cat. no. ab3347; Abcam; 1:1,000) primary antibodies, followed by incubation with horseradish peroxidase-conjugated secondary antibody (1:3,000; cat. no. 7074; Cell Signaling Technology, Inc.) at  $37^\circ\text{C}$  for 30 min. Immunostaining was visualized using DAB chromogen by incubation for 1 min at room temperature (cat. no. D4293; Sigma-Aldrich; Merck KGaA) and sections were counterstained with Mayer's hematoxylin for between 30 sec and 1 min at room temperature.

**Western blot analysis.** Total proteins and nucleoproteins were extracted using Total Protein Extraction kit (Beyotime Institute of Biotechnology) and Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Institute of Biotechnology) for homogenized myocardial tissue samples. Protein concentrations were measured using a bicinchoninic acid assay. The equivalent of 60  $\mu\text{g}$  protein was separated on a 12% SDS-PAGE and transferred to 0.22  $\mu\text{m}$  polyvinylidene fluoride membranes. Next, the membranes were blocked with 5% fat-free milk for 2 h at  $4^\circ\text{C}$  and incubated with the relevant

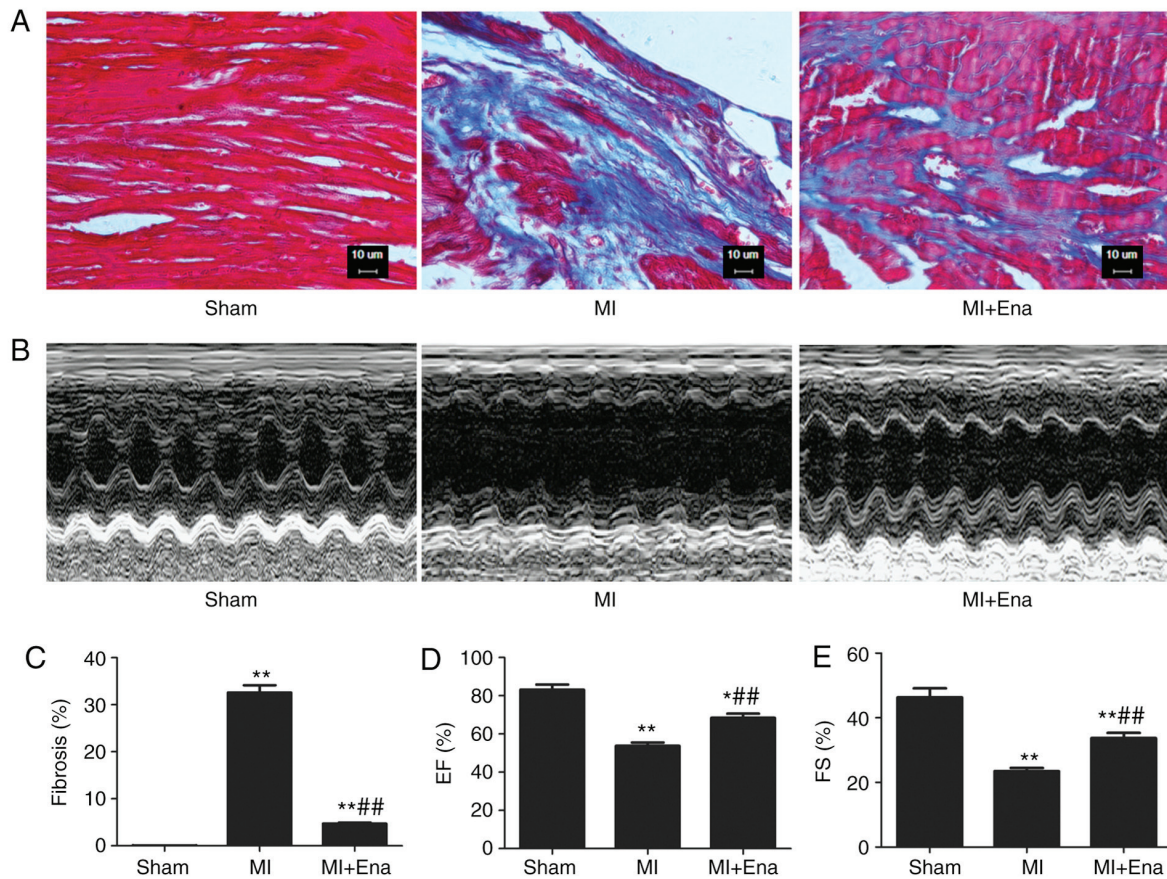


Figure 1. Enalapril decreases myocardial fibrosis and increases cardiac systolic function in the hearts of mice following MI. (A) Representative Masson's trichrome images of sections from the ischemic area of MI mice treated with enalapril or control. Scale bar, 10  $\mu$ m. Magnification x400. (B) Representative images produced by echocardiography from different groups of mice (sham, MI and MI treated with enalapril). (C) Fibrosis in sham, MI and MI+Ena groups. (D) The EF and (E) FS at the end of treatment with enalapril or control. \* $P < 0.05$  and \*\* $P < 0.01$  vs. sham group. ### $P < 0.01$  vs. MI group. MI, myocardial infarction; EF, ejection fraction; FS, fraction shortening; Ena, enalapril.

protein antibodies overnight at 4°C, including antibodies against poly (ADP-ribose) polymerase (PARP)-1 (1:1,000; cat. no. 9532; Cell Signaling Technology, Inc.), caspase-9 (1:1,000; cat. no. 9508; Cell Signaling Technology, Inc.), cleaved caspase-9 (1:1,000; cat. no. 7237; Cell Signaling Technology, Inc.), caspase-3 (1:1,000; cat. no. 9665; Cell Signaling Technology, Inc.), cleaved caspase-3 (1:1,000; cat. no. 9694; Cell Signaling Technology, Inc.), 78 kDa glucose-regulated protein (GRP78; 1:500; cat. no. sc-13968; Santa Cruz Biotechnology, Inc.), activating transcription factor 6 (ATF6; 1:1,000; cat. no. 65880; Cell Signaling Technology, Inc.), Bcl-2 (1:1,000; cat. no. 3498; Cell Signaling Technology, Inc.), Bax (1:1,000; cat. no. 14796; Cell Signaling Technology, Inc.), TAK1 (1:1,000; cat. no. ab109526, Abcam), phosphorylated TAK1 (1:1,000; cat. no. 9339; Cell Signaling Technology, Inc.), NFAT3 (1:1,000; cat. no. ab3347; Abcam),  $\beta$ -actin (1:1,000; cat. no. 3700; Cell Signaling Technology, Inc.) and Lamin B1 (1:1,000; cat. no. 13435; Cell Signaling Technology, Inc.). The membranes were washed with TBS containing 1% Tween-20 and incubated with horseradish peroxidase-conjugated second antibody (1:3,000; cat. no. 7074; Cell Signaling Technology, Inc.) for 2 h at room temperature. ECL was used as chromogenic method. Signals were visualized using a ChemiDICTMXRS + Imaging System (Bio-Rad Laboratories,

Inc.) and the band density was quantified using the Image Lab™ 5.2 software (Bio-Rad Laboratories, Inc.). The amount of protein was analyzed using Image-Pro Plus (version 6.0; Media Cybernetics, Inc.) and normalized to the respective controls.

**Statistical analysis.** All data are presented as the mean  $\pm$  standard deviation from at least 3 independent experiments. All statistical analyses were performed using the SPSS 19.0 statistical software package (IBM Corp.). One-way ANOVA was used to compare data from multiple groups. For data with homogeneity of variance, the Least Significant Difference post-hoc test was used, and the Dunnett's T3 post-hoc test was performed for those data with irregular variance.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Effect of enalapril on the MI-evoked fibrotic response and cardiac systolic function in vivo.** To evaluate the protective effect of enalapril on MI, the effects of enalapril-treatment on myocardial fibrosis in mice were investigated and the results are presented in Fig. 1A. Myocardial fibrosis was not



Table I. Comparison of myocardial fibrosis, EF and FS data between groups.

Groups	Myocardial fibrosis (%)	Ejection fraction (EF) (%)	Fraction shortening (FS) (%)
Sham	0.06±0.05	83.17±5.40	46.32±5.64
MI	32.6±3.44 <sup>b</sup>	53.76±4.07 <sup>b</sup>	23.48±2.25 <sup>b</sup>
MI+Ena	4.72±0.50 <sup>b,c</sup>	68.43±5.36 <sup>a,c</sup>	33.73±3.86 <sup>b,c</sup>
F-value	385.45	38.96	36.54
P-value	<0.01	<0.01	<0.01

EF, Ejection fraction; FS, fraction shortening; Ena, enalapril; MI, myocardial infarction. <sup>a</sup>P<0.05 and <sup>b</sup>P<0.01 vs. Sham group. <sup>c</sup>P<0.01 vs. MI group.

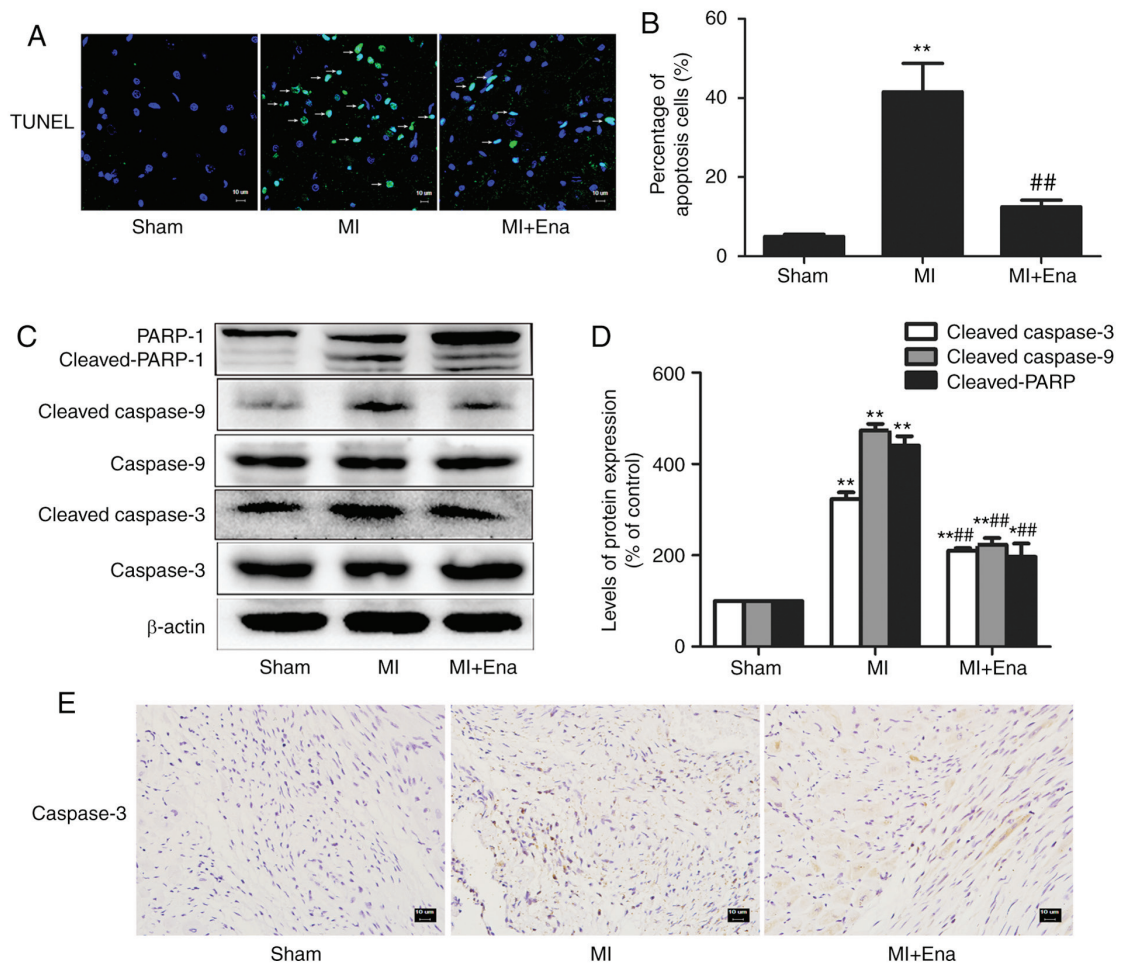


Figure 2. Enalapril decreases myocardial apoptosis in MI mice. (A) Representative TUNEL immunofluorescence images in different groups of mice. The white arrows indicate the TUNEL-positive cells. Scale bar, 10  $\mu$ m. Magnification, x400. (B) Percentages of apoptosis-positive cells. (C) Endoplasmic reticulum stress-associated and mitochondrial dysfunction-associated proteins were detected by western blot analysis. Protein levels of cleaved PARP, cleaved caspase-3 and cleaved caspase-9 in the hearts of control mice, MI mice and MI mice treated with enalapril. (D) Optical density analysis of cleaved PARP, cleaved caspase-3 and cleaved caspase-9 in the heart tissues. (E) Immunohistochemical staining for cleaved caspase-3 in the hearts of control mice, MI mice and MI mice treated with enalapril. The data are presented as the mean  $\pm$  standard deviation (n=6). \*P<0.05 and \*\*P<0.01 vs. sham group. \*\*\*P<0.01 vs. MI group. MI, myocardial infarction; PARP, poly (ADP-ribose) polymerase; Ena, enalapril.

observed in the control group. Compared with the MI group, the MI+Ena group presented with less myocardial fibrosis (Fig. 1A and C). Cardiac systolic function was also observed and measured by echocardiography, ejection fraction (EF) measurement and fraction shortening (FS) measurement

(Fig. 1B, D and E). Cardiac systolic function was normal in the control group (EF, 83.18%; FS, 46.33%). Compared with the MI group (EF, 53.76%; FS, 23.48%), the MI+Ena group (EF, 68.43%; FS, 33.73%) demonstrated a significant improvement in cardiac systolic function, suggesting that

Table II. Comparison of myocardial apoptosis, cleaved caspase-3, cleaved caspase-9 and cleaved PARP between different groups.

Data	Groups			F-statistic	P-value
	Sham	MI	Mi-Ena		
Myocardial fibrosis (%)	5.0±1.0	41.56±12.42 <sup>b</sup>	12.54±2.85 <sup>c</sup>	20.53	<0.01
Cleaved caspase-3	100±0.1	323.33±25.17 <sup>b</sup>	210±10.0 <sup>b,c</sup>	152.9	<0.01
Cleaved caspase-9	100±0.1	473.33±25.17 <sup>b</sup>	223.33±25.17 <sup>b,c</sup>	256.96	<0.01
Cleaved PARP	100±0.1	440.33±36.1 <sup>b</sup>	196.67±50.33 <sup>a,c</sup>	72.03	<0.01

PARP, poly (ADP-ribose) polymerase; Ena, enalapril; MI, myocardial infarction. <sup>a</sup>P<0.05 and <sup>b</sup>P<0.01 vs. Sham group. <sup>c</sup>P<0.01 vs. MI group.

Table III. Comparison of GRP78, ATF6, Bax/Bcl-2, p-TAK1/TAK1 and NFAT3 expression between different groups.

Data	Groups			F-statistic	P-value
	Sham	MI	Mi-Ena		
GRP78	100±0.15	331.67±27.54 <sup>a</sup>	168.33±16.1 <sup>a,d</sup>	125.31	<0.01
ATF6	100±0.1	235.0±15.0 <sup>a</sup>	140.0±10.0 <sup>a,d</sup>	132.99	<0.01
Bax/Bcl-2	1.00±0.001	2.99±0.44 <sup>a</sup>	0.64±0.03 <sup>b,c</sup>	74.34	<0.01
p-TAK1/TAK1	1.00±0.003	1.45±0.21 <sup>a</sup>	1.99±0.25 <sup>b,c</sup>	20.91	<0.01
Intracytoplasmic NTFA3	100±0.29	186.67±15.28 <sup>a</sup>	313.33±32.15 <sup>a,c</sup>	81.67	<0.01
NFAT3 in the nucleus	100±0.1	157.82±12.05 <sup>a</sup>	221.34±18.75 <sup>a,c</sup>	66.63	<0.01

GRP78, 78 kDa glucose-regulated protein; ATF6, activating transcription factor; p, phosphorylated; TAK1, transforming growth factor-activated kinase 1; NTFA3, nuclear factor of activated T cells 3; Ena, enalapril; MI, myocardial infarction. <sup>a</sup>P<0.05 and <sup>b</sup>P<0.01 vs. Sham group. <sup>c</sup>P<0.05 and <sup>d</sup>P<0.01 vs. MI group.

treatment with enalapril decreased myocardial fibrosis and increased cardiac systolic function *in vivo*. The specific myocardial fibrosis, EF and FS data are presented in Table I.

*Enalapril decreases myocardial apoptosis in MI mice.* To determine the role of enalapril in cardiac protection, enalapril was injected into the mouse abdominal cavity at 1 week after MI for 3 weeks. Myocardial apoptosis was detected via TUNEL staining in the infarct and border areas. As presented in Fig. 2A and B, there were no apoptosis-positive cells in the sham group. The number of TUNEL-positive cells increased significantly at 4 weeks after MI and a protective effect was observed in the enalapril-treatment group.

To additionally confirm the protective effect of enalapril, the expression levels of proteins involved in the caspase pathway were examined in the heart following MI by western blotting. The levels of cleaved PARP, cleaved caspase-3 and cleaved caspase-9 decreased significantly following treatment with enalapril compared with the MI group (Fig. 2C and D). In addition, fewer cleaved caspase-3-positive cells were identified in the control group via immunohistochemical analysis. The number of cleaved caspase-3-positive cells increased significantly in the MI group, and the enalapril-treatment group exhibited protective effects (Fig. 2E). These results indicated that enalapril-treatment had a cardioprotective effect and significantly decreased activation of the caspase pathway. The complete data from the myocardial cell

apoptosis staining and western blot analysis assays are presented in Table II.

*Enalapril inhibits ER stress and mitochondrial dysfunction in MI mice.* To confirm whether the cardioprotective effect of enalapril is associated with ER stress and mitochondrial dysfunction, the expression levels of ER stress- and mitochondrial dysfunction-associated proteins were measured. Western blot analysis indicated that the protein expression levels of GRP78 and ATF6 were significantly upregulated in the hearts of MI mice compared with the sham group. Furthermore, enalapril treatment inhibited the activation of ER stress-associated proteins in the hearts of MI mice (Fig. 3A). In addition, the western blot analysis results suggested that enalapril inhibited the upregulation of the mitochondrial dysfunction-associated proteins Bax and Bcl-2, which were induced by MI (Fig. 3B). To further understand the mechanism underlying the effect of enalapril on MI, activation of TAK1/NFAT downstream signaling was also analyzed by western blot analysis. As expected, enalapril treatment increased the phosphorylation of TAK1 and NFAT3 in the heart tissues of MI mice compared with the controls (Fig. 3C and D). Furthermore, immunohistochemistry confirmed the protein expression of NFAT3 (Fig. 3E), which was consistent with the western blot analysis data. In addition, compared with the MI group, enalapril treatment increased the nuclear transfer of NFAT3 (Fig. 4). In summary,

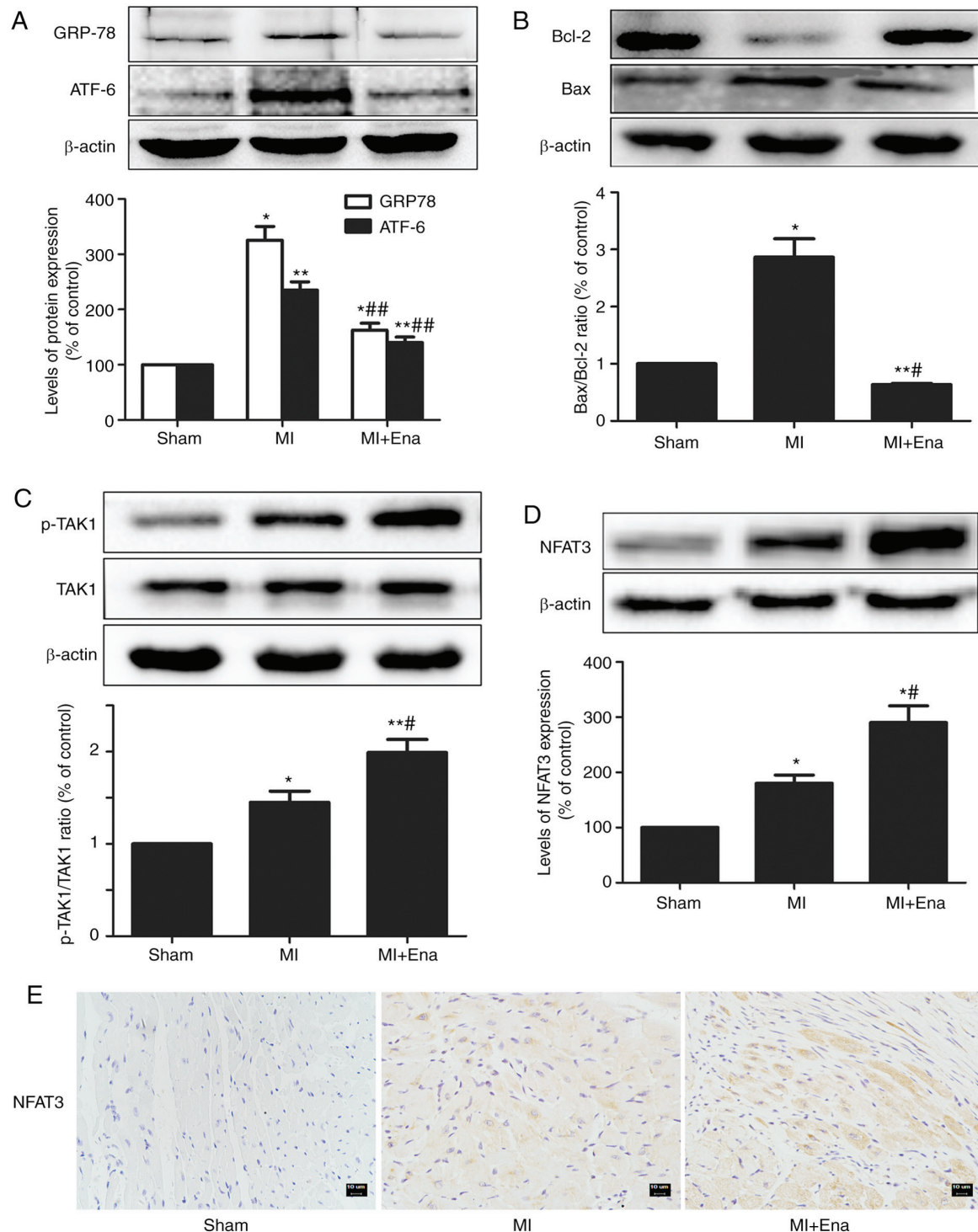


Figure 3. Enalapril inhibits ER stress and mitochondrial dysfunction in MI mice. (A) Protein expression levels and quantitative analysis of GRP78 and ATF6. (B) Protein expression levels and quantitative analysis of Bcl-2 and Bax. (C) Protein expression levels and quantitative analysis of TAK1 and p-TAK1. (D) Protein expression levels and quantitative analysis of intracytoplasmic NFAT3. (E) Immunohistochemical staining for NFAT3 in the hearts of control mice, MI mice and MI mice treated with enalapril. Magnification, x400. The data are presented as the mean  $\pm$  standard deviation (n=6). \* $P<0.05$  and \*\* $P<0.01$  vs. sham group. # $P<0.05$  vs. the MI group. Ena, enalapril; ER, endoplasmic reticulum; MI, myocardial infarction; GRP78, 78 kDa glucose-regulated protein; ATF6, activating transcription factor 6; TAK1, transforming growth factor- $\beta$ -activated kinase 1; p, phosphorylated; NFAT3, nuclear factor of activated T cells 3.

these results suggested that the protective role of enalapril in MI was associated with the inhibition of ER stress, mitochondrial dysfunction and TAK1/NFAT signaling pathway. The complete western blot analysis data are summarized in Table III.

## Discussion

The present study demonstrated that enalapril inhibited cardiomyocyte apoptosis, including via the inhibition of ER stress and mitochondrial dysfunction, and prevented



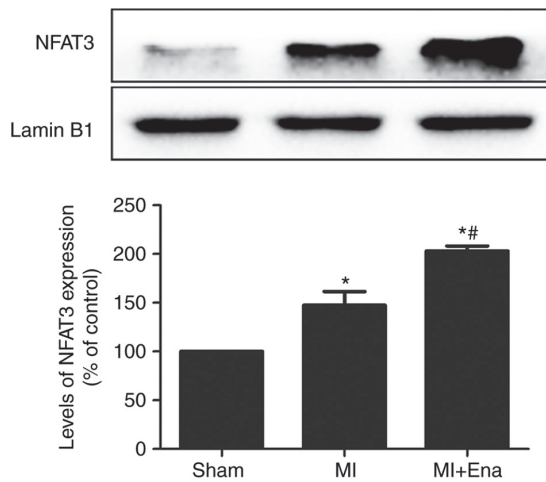


Figure 4. Nuclear protein expression levels and quantitative analysis of NFAT3 in MI mice. The data are presented as the mean  $\pm$  standard deviation (n=6). \*P<0.05 vs. sham group; #P<0.05 vs. MI group. Ena, enalapril; MI, myocardial infarction; NFAT3, nuclear factor of activated T cells 3.

ventricular structural remodeling by activating the TAK1/NFAT pathway *in vivo*.

The morbidity and mortality rates of MI are high worldwide. Following infarction, myocardial injury is followed by apoptosis, inflammation and remodeling. The apoptosis of myocardial cells is an important inhibiting factor in myocardial recovery. Furthermore, it is a general consensus that MI activates the RAS, with high expression of Ang II, which is followed by ventricular remodeling. Concomitantly, calcium/calmodulin-dependent protein kinase II, which promotes cardiomyocyte apoptosis *in vivo*, is activated by Ang II following MI (23,24). It has been demonstrated that Ang II is significantly increased in the infarct region of heart tissue (25). Enalapril, an angiotensin-converting enzyme inhibitor, inhibits the formation of Ang II and decreases the damage caused by Ang II. Several studies have also indicated that enalapril reduced the risk of MI (7), inhibited remodeling during the healing process after MI (26) and reduced mortality associated with cardiovascular events (6). Enalapril treatment also prevents apoptosis (9). The present study demonstrated that treatment with enalapril for 3 weeks prevented ventricular remodeling and apoptosis following MI.

TAK1, a member of the mitogen-activated protein kinase kinase family, was originally identified as a candidate positive mediator of TGF- $\beta$  signal transduction (27). The TGF- $\beta$ 1-TAK1-p38 mitogen activated protein kinase pathway is activated in post-MI left ventricular remodeling in rats, and the mRNA and protein levels of TAK1 are increased following MI (28). Furthermore, TAK1 is activated when cardiac progenitor cell apoptosis is inhibited *in vitro* and prevents heart failure progression following MI (29). Previous studies have proposed that TAK1 exerts a regulatory function in cell viability (30,31). Fauster *et al* (32) also verified that TAK1 is a novel and direct target of ponatinib, an inhibitor of necroptosis. It was confirmed that TAK1 activity is cardioprotective in muramyl dipeptide (MDP)-treated hearts

in a mouse model of ischemia/reperfusion. Furthermore, the TAK1 inhibitor 5Z-7-oxozeanol abolished the decrease in infarct size observed in MDP-treated hearts (33). TAK1 regulates NF- $\kappa$ B signaling pathways that serve key roles in development, cell survival and immune responses (34). The evolutionarily conserved signaling intermediate in Toll pathways protein forms a signaling complex with TAK1 and TRAF6 through specific molecular interactions, where TAK1 may affect downstream cascade signaling for the activation of NF- $\kappa$ B (35). Therefore, the expression of NF- $\kappa$ B may be closely associated with the change in TAK1 expression. However, the specific association between NF- $\kappa$ B and TAK1 in the treatment of MI with enalapril remains unknown.

The NFAT transcription factor family consists of 5 members: NFATcytoplasmic (c)1, NFATc2, NFATc3, NFATc4 and NFAT5 (36). NFATc proteins are dephosphorylated and localize to the nucleus following treatment with calcineurin (36). A number of studies have reported that NF-ATc4 activation is increased following MI (37,38). Furthermore, NFAT mediates cardioprotection in MI (39). Notably, TAK1 has been demonstrated to activate several signaling pathways, including calcineurin-NFAT signaling (40).

The present study also had certain limitations: Whether the treatment effect of enalapril was associated with the time intervals of enalapril treatment, or whether enalapril inhibited apoptosis via TAK1 through (5Z)-7-Oxozeanol, which is a specific inhibitor of TAK1, are issues requiring additional study. To the best of our knowledge, the underlying mechanism of the effect of enalapril on MI remains incompletely understood. However, the results of the present study suggested novel possibilities for the mechanism of enalapril treatment.

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#### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

#### Authors' contributions

XR mainly completed statistical analysis of data and article modification; DHG performed the writing of this manuscript, TUNEL assay and animal model experiments; LLY performed the Western blot and Masson's trichrome (MT) staining; LL performed the echocardiographic measurements; MPC and HTL are corresponding authors and designed this study.

## Ethics approval and consent to participate

All animal experiments were approved by the Animal Ethics Committee of Wenzhou Medical University (approval no. wydw2017-0007) and were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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