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# Perindopril inhibits myocardial apoptosis in mice with acute myocardial infarction through TLR4/NF-κB pathway

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**Abstract. – OBJECTIVE: To explore the anti-apoptotic effect of perindopril on myocardial cells in mice with acute myocardial infarction (AMI).**

**MATERIALS AND METHODS: A total of 48 mice were randomly divided into 4 groups before intervention, namely sham operation group (Sham group, n=12), AMI group (n=12), 1.5 mg/kg perindopril treatment group (Perindopril group, n=12), and 1.5 mg/kg perindopril treatment and Toll-like receptor-4 (TLR4) knockout group (TLR4-/-Perindopril group, n=12). Mice in the control group and AMI group were gavaged with normal saline, and those in the Perindopril group and TLR4-/-Perindopril group were gavaged with perindopril for 7 d. On the 4th day after drug administration, mice in the AMI group, Perindopril group and TLR4-/-Perindopril group were subjected to the ligation of the anterior descending coronary artery to induce AMI, and those in the Sham group underwent the same operation, but had a loose knot at the anterior descending coronary artery. At 24 h after the above operation, color echocardiography was performed on mice to observe changes in cardiac function. Then, the mice were sacrificed. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP)-biotin nick end labeling (TUNEL) assay was carried out to determine myocardial apoptosis. Immunohistochemistry and Western blotting technique were employed to detect the protein expression levels of B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax), TLR4 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p50 in infarction zones. The messenger ribonucleic acid (mRNA) expression levels of TLR4 and NF-κB p50 in infarction zones were measured *via* Reverse Transcription-Polymerase Chain Reaction (RT-PCR).**

**RESULTS: Perindopril could significantly reduce the number of apoptotic myocardial cells after AMI. Mouse echocardiography showed that ejection fraction (EF), left ventricular fractional shortening (FS), left ventricular end-systolic diameter (LVESD) and left ventricular end-diastolic diameter (LVEDD) of AMI mice in the Perindopril groups were markedly superior to those in the AMI group. AMI mice in the Perindopril group had decreased expression levels of Bax protein and TLR4 and NF-κB p50 mRNA and protein, as well as the Bax/Bcl-2 ratio. Knockout of TLR4 attenuated the effect of perindopril in alleviating myocardial apoptosis after AMI.**

**CONCLUSIONS: Perindopril inhibits myocardial apoptosis in mice with AMI through the TLR4/NF-κB pathway.**

*Key Words:*
Perindopril, TLR4, NF-κB, Acute myocardial infarction, Apoptosis.

## Introduction

Cardiovascular disease is the leading killer in industrialized countries, which will rank the first among fatal diseases in developing countries by 2020[1]. Acute myocardial infarction (AMI) has the highest incidence and mortality rates in cardiovascular diseases. As an important event in the development of AMI, myocardial apoptosis is a major component of myocardial cell death in the acute ischemic phase[2]. Apoptosis is also known as programmed cell death, which is a mode of active cell death controlled by genes and plays a


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very important role in normal growth and the development of various diseases. After AMI, myocardial necrosis and apoptosis are observed. A study revealed that the size of AMI depended on myocardial apoptosis and necrosis, the myocardial infarction size caused by myocardial apoptosis accounts for 86% of the total myocardial infarction size, and that due to myocardial necrosis, accounts for 14%[3]. The reduction in the number of myocardial cells is one of the mechanisms by which myocardial infarction leads to heart failure, and the inhibition of myocardial apoptosis after myocardial infarction can improve left ventricular remodeling and cardiac function[4].

Apoptosis, directly controlled by intracellular apoptosis-related genes, is a mode of cell death that is regulated by extracellular signals. Animal experiments[3,5] and autopsies have found that myocardial apoptosis occurs at different stages after myocardial infarction; it can be mediated by different death signals and may be regulated and controlled by different genes.

Toll-like receptor-4 (TLR4) is a class of innate immune receptors that can bind to corresponding ligands to further activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)[6]. NF-κB is a widely-expressed transcription factor affecting immune regulation, cell migration and apoptosis. An experiment proved that TLR4 messenger ribonucleic acid (mRNA) expression is detected in myocardial cells in neonatal rats, which is increased after stimulation with lipopolysaccharide (LPS), Interleukin - 1β (IL-1β) and Interferon-γ (IFN-γ), activating NF-κB, and declines when NF-κB is blocked by PDTC (a specific NF-κB blocker)[7]. The B-cell lymphoma 2 (Bcl-2) gene, an abbreviation of the B cell lymphoma/leukemia-2 gene, is the first gene that is confirmed to be able to inhibit apoptosis. Bcl-2 protein can suppress apoptosis induced by different factors. Matsushita et al[8] found that activated NF-κB is capable of mediating apoptosis through Bcl-2. The above experiments have fully proven that the TLR4 signaling pathway is associated with myocardial apoptosis, and apoptosis can be repressed by blocking different signaling molecules of the TLR4 signaling pathway through different methods.

Recent experimental studies[9,10] have manifested that the application of certain medications before ischemia (pretreatment) can relieve ischemia-reperfusion injury and decrease the infarct size caused by AMI. Perindopril is an angiotensin-converting enzyme inhibitor (ACEI) drug commonly used for hypertension[11]. In recent years, Douillette et al[12] have discovered that angiotensin II (Ang II) can activate NF-κB to cause the inflammation of the cardiovascular system, and the inhibition of angiotensin II expression can reduce the activity of NF-κB. In addition, ACEI drugs can suppress NF-κB activity in myocardial cells in rats with myocardial infarction[13]. This experiment aims to observe the effect of perindopril pretreatment on experimental AMI and myocardial apoptosis in mice.

## Materials and Methods

### Animals and Reagents

A total of 36 C57BL/6J mice aged 8 weeks and weighing (18.2 ± 0.5) g and 12 TLR4 knockout mice aged 8 weeks and weighing (20.4 ± 0.4) kg, both with normal nutritional status and mental status, were provided by the Laboratory Animal Center of Beijing University of Chinese Medicine. This study was approved by the Animal Ethics Committee of Beijing University of Chinese Medicine Animal Center. Bcl-2-associated X protein (Bax), Bcl-2, TLR4, NF-κB p50 antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA). TLR4 and NF-κB p50 primers were bought from Shanghai Bioengineering Co., (Shanghai, China). RNA Reverse Transcription kit and quantitative Polymerase Chain Reaction (qPCR) kit were purchased from TaKaRa (Otsu, Shiga, Japan).

### Model Establishment and Grouping

Before the operation, mice were weighed, intraperitoneally injected with avertin solution (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 0.40-0.75 mg/g for anesthesia, and fixed on a rat plate in supine position. Then, the skin of the neck and precordial region was disinfected with 70% alcohol, and the hair was shaved. After that, the skin of the neck was scissored and the muscle was bluntly separated, followed by tracheal intubation and connection of a small animal ventilator (positive pressure ventilation 2-3 mL/cycle, frequency 20 cycles/min). Thereafter, the skin of the precordial region was scissored, the muscle was bluntly separated and the chest wall was cut using a pair of scissors at the space between the third and fourth ribs. For 20 mice in the AMI group (group A), a 7-0 suture needle with suture was used to ligate the left anterior descending coronary artery (coronary artery) at 3-4 mm be-

low the left auricle. For another 10 mice served as the Sham group (group B), the needle passed through the left anterior descending coronary artery, but the artery was not ligated. Then, the thoracic cavity was closed along the third and fourth ribs, the chest muscles were sutured layer by layer, and finally the chest skin was continuously sewn up. After that, the chest was squeezed to remove thoracic gas to recruit the lungs, the ventilator was removed and the neck skin was sewn up. Lastly, mice were put on a 40°C insulation platform for regaining consciousness.

### Echocardiography and Specimen Processing

The cardiac function of mice was determined at 24 h after operation using a Vevo 770 ultrasound system and a 30 MHz high-frequency probe. Mice were anesthetized by intraperitoneally injecting with avertin solution at a dose of 0.40-0.75 mg/g, and the heart rate after anesthesia was controlled at 350-450 beats/min. The hair in the precordial region was shaved and mice were fixed on the built-in hot plate of the ultrasound system in supine position to keep the body temperature constant. Then, an appropriate amount of ultrasonic coupling agent was applied to the precordial region; the 30 MHz probe of the ultrasound system was used to collect B-Mode images of the parasternal long axis view and apical four chamber view and record the electrocardiogram. The M-shaped section image of the parasternal left ventricular short axis was obtained at the level of chordae tendineae mitral valve. Left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic period (LVEDD), left ventricular diastolic posterior wall thickness, left ventricular diastolic volume and left ventricular ejection fraction (LVEF) were measured. For different individuals, the parameter settings of the Vevo 700 ultrasound system were the same. For each measurement index, three consecutive cardiac cycles were selected, and the average was taken. After echocardiography, mice were killed, the chest was opened, and the heart was taken out. Then, the residual blood was washed off with 4°C Phosphate-Buffered Saline (PBS; Gibco, Grand Island, NY, USA), followed by drying with filter papers for further treatment.

### Immunohistochemistry

Paraffin-embedded heart tissue sections were deparaffinized with xylene, dehydrated with graded alcohol and incubated with warm deionized water containing 0.3% $H_20_2$ (Sigma-Aldrich, St. Louis, MO, USA) for 30 min. After the endogenous peroxide was eliminated, the sections were blocked with serum and added with primary antibodies for incubation at 4°C overnight. On the next day, the sections were incubated with immunoglobulin G (IgG) antibody-horseradish peroxidase (HRP; Abcam, Cambridge, MA, USA) and dropwise added with the mixture prepared in the biotin and ABC kit (TaKaRa, Otsu, Shiga, Japan) for culture, followed by color development with diaminobenzidine (DAB; Solarbio, Beijing, China) for 10 min. Thereafter, sections were counterstained with hematoxylin, washed, dehydrated and permeabilized. Lastly, the sections were observed using an optical microscope.

### Terminal Deoxynucleotidyl Transferase-Mediated Deoxyuridine Triphosphate (dUTP)-Biotin Nick End Labeling (TUNEL)

Paraffin-embedded tissue sections were deparaffinized with xylene, dehydrated with graded alcohol and treated with Proteinase K working solution for 15-30 min at 21-37°C or added with a cell-permeable solution for 8 min of reaction. TUNEL (Roche, Basel, Switzerland) reaction mixture was prepared. 50 μL of $TdT^+$ 450 μL of fluorescein-labeled dUTP solution was added and mixed in the treatment group, and only 50 μL of fluorescein-labeled dUTP solution was added in the negative control group. After slides were dried, 50 μL of TUNEL reaction mixture (only 50 μL of fluorescein-labeled dUTP solution was added in the negative control group) was added onto specimens, and slides were covered with coverslips or sealing films, followed by reaction in a dark and humid chamber at 37°C for 60 min. After the slides were dried, 50 μL of converter-POD was added onto specimens, slides were covered with coverslips or sealing films, followed by reaction in the dark and humid chamber at 37°C for 30 min. Thereafter, the sections were added with 50-100 μL of DAB substrate for reaction at 15-25°C for 10 min. After that, they were counterstained with hematoxylin or methyl green for a few seconds and rinsed with tap water immediately, dehydrated with graded alcohol, permeabilized with xylene and mounted with neutral gum. A drop of PBS or glycerol was added, and the optical microscope was employed for counting (200-500 cells) and photographing. Positive cells were counted, and apoptotic index (AI) = number of positive cells/total number of

cells × 100. The mean calculated in each group was used as a representative value.

### Western Blotting

Collected tissues were ground in liquid nitrogen, diluted with normal saline and placed on ice. Then, the supernatant was collected and centrifuged at 4°C for 5 min, and the supernatant was discarded. After that, the precipitate was re-suspended in radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) lysis buffer containing phenylmethanesulfonyl fluoride (PMSF; R & D Systems, Minneapolis, MN, USA), lysed and centrifuged at 4°C and 16000 g for 15 min, and the supernatant was taken for protein quantification. Thereafter, the protein was added with loading buffer, heated for denaturation, subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a membrane. The membrane was blocked with 5% skim milk for 2 h, added with primary antibodies for incubation at 4°C overnight and washed with Tris-Buffered Saline and Tween 20 (TBST; Sigma-Aldrich, St. Louis, MO, USA) 3 times (10 min/time), followed by incubation with corresponding secondary antibodies at room temperature for 1 h and washing with TBST 3 times (10 min/time). Lastly, electrochemiluminescence (ECL; Thermo Fisher Scientific, Waltham, MA, USA) assay was performed to detect the protein expression in different samples.

### Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

After taking an appropriate amount of tissue samples, TRIzol (Invitrogen, Carlsbad, CA, USA) was added for lysis at room temperature for 5 min. Then, tissue lysis solution was added with 1/5 volume of chloroform and shaken well until the solution was fully emulsified without stratification, followed by still standing at room temperature for 5 min. After that, the solution was centrifuged at 4°C and 12000 g for 15 min, and the supernatant was collected to an Eppendorf tube (EP; Eppendorf, Hamburg, Germany). Then, the same volume of isopropanol was added and mixed, followed by still standing at room temperature for 10 min, centrifugation at 4°C and 12000 g for 10 min and discard of the supernatant. Thereafter, the precipitate was washed with 75% ethanol and centrifuged for 5 min, and the ethanol was removed. The precipitate was dried for 2-3 min and dissolved with 20 μL of sterile diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China), followed by measurement of RNA concentration. After that, the Reverse Transcription was carried out according to the instructions of the Reverse Transcription kit (Invitrogen, Carlsbad, CA, USA). PCR reaction system (20 μL in total): 10 μL of TB Green Premix Ex TaqII (2×), 0.4 μL of ROX Reference Dye or Dye II (50×), 0.8 μL of forward primer (10 μmol/L), 0.8 μL of reverse primer (10 μmol/L), 2 μL of cDNA solution and 6 μL of sterilized $ddH_2O$. PCR cycle parameters: pre-denaturation at 95°C for 30 s, at 95°C for 5 s, at 60°C for 34 s and fluorescence collection at 60°C, for a total of 40 cycles. Data were collected using the Applied Biosystems 7500 Fast Real Time-PCR System (Foster City, CA, USA). With GAPDH as a housekeeping gene, the relative expression level was calculated by the $2^{-\Delta\Delta Ct}$ method. The following primers are used to detect TLR4 (forward primer 5′-ACAAGGCATGGCATGGCTTACAC-3′, reverse primer 5′-TGTCTCCACAGCCACCAGATTCTC-3′), NF-kB P50 (forward primer 5′-GACACGACAGAATCCTCAGCATCC-3′, reverse primer 5′-CCACCAGCAGCAGCAGACATG-3′), and GAPDH (forward primer 5′-GCCTCGTCCCGTAGACAAAA-3′, reverse primer 5′-GATGGGCTTCCCGTTGATGA-3′).

### Statistical Analysis

Data were expressed as mean ± standard deviation and analyzed through a paired or unpaired *t*-test. Comparison between groups was made using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ suggested that the difference was statistically significant. Data were analyzed using the Statistical Product and Service Solutions (SPSS 20.0) statistical software (SPSS Inc., Chicago, IL, USA), and then GraphPad software (Version X; La Jolla, CA, USA) was used for plotting.

## Results

### Perindopril Improved Cardiac Function in Mice With AMI and Reduced the Number of Apoptotic Myocardial Cells

In this experiment, a total of 36 mice underwent coronary ligation, among which 28 mice survived after 24 h after operation, and the remaining 12 mice received sham operation and were included in the Sham group. Echocardiography was performed for mice in each group

after establishing models of AMI *via* ligation, and relevant data suggested that the ejection fraction (EF), left ventricular fractional shortening (FS), left ventricular end-systolic diameter (LVESD) and left ventricular end-diastolic diameter (LVEDD) of mice were decreased in the AMI group compared with those in the Sham group ($p<0.05$), while the echocardiogram data were evidently higher in the Perindopril group than those in the AMI group ($p<0.05$); TLR4-/-Perindopril group had decreased echocardiogram data compared to the Perindopril group (Table I), indicating that the cardiac function of AMI mice receiving perindopril treatment is better than that of those taking no perindopril. Myocardial apoptosis in mice was detected *via* TUNEL assay. Apoptotic cells are positive for TUNEL staining, with brownish-yellow nuclei. In this work, a small number of apoptotic myocardial cells was found in the control group (Figure 1). A large number of apoptotic myocardial cells was observed in infarction zones in the AMI group, which was significantly increased compared with that in the control group ($p<0.05$; Figure 1). The Perindopril group exhibited markedly lowered the number of apoptotic myocardial cells in comparison with the AMI group ($p<0.05$), while such a decrease was counteracted by TLR4 knockout ($p<0.05$; Figure 1).

### Perindopril Reduced the Expression Levels of Myocardial Bax and Bcl-2 in Infarction Zones of Mice With AMI

Immunohistochemistry was employed to measure the protein expression levels of Bax and Bcl-2. The cells positive for staining are brown or brownish-yellow and localized in the cytoplasm. Image-Pro Plus 6.0 (Version X; Media Cybernetics, Silver Springs, MD, USA) was utilized for calculation of the average optical density in each group and protein quantification. The more the protein was and the darker the color was, the greater the average optical density value would be. We found that the Bax protein expression was detected in all groups. The Bax protein expression level was significantly higher in the AMI group than that in the control group ($p<0.05$), and it was overtly reduced after perindopril treatment (Figure 2A, 2B). Bcl-2 protein is also expressed in the cytoplasm. Bcl-2 protein expression was observed in each group ($p<0.05$), which was overtly elevated in the AMI group and Perindopril group compared with that in the control group ($p<0.05$). There was no significant difference in Bcl-2 protein between the AMI group and Perindopril group (Figure 2C, 2D). However, the protein expression levels of Bax and Bcl-2 in TLR4-/-Perindopril group were increased compared with those in the Perindopril group. The above results imply that the treatment with perindopril can reduce the gene and protein expression levels of Bax (a myocardial apoptosis gene) in infarction zones in mice with AMI, and TLR4 knockout can attenuate this effect of perindopril to some extent.

### Perindopril Lowered the Expression of Myocardial TLR4/NF-κB in Infarction Zones in Mice With AMI

The protein expression of TLR4 in infarction zones was detected *via* immunohistochemistry. TLR4 is expressed in the cytoplasm and brownish-yellow. This study discovered that TLR4 protein expression was observed in all groups (Figure 3A, 3C). The color of stained TLR4 protein was relatively light in the control group and dark in the AMI group. QPCR and Western blotting results revealed that TLR4 mRNA and protein expression levels were higher in the AMI group than those in the control group (Figure 3B, 3D; $p<0.05$), and they were markedly reduced in the Perindopril group compared with those in the AMI group ($p<0.05$).Immunohistochemistry was used to detect the expression of NF-κB p50 protein in myocardial tissues of mice with myocardial infarction, and the results showed that the expression of NF-κB p50 protein was found in all groups (Figure 4A, 4C).

**Table I.** Changes of heart volume and function in the mice ($\bar{x} \pm s$).

| Group | Number | EF (%) | FS (%) | LVEDD (μm) | LVESD (μm) |
|---|---|---|---|---|---|
| Sham | 12 | 78.38 ± 2.36 | 51.40 ± 4.33 | 34.74 ± 1.36 | 14.47 ± 4.25 |
| AMI | 8 | 45.81 ± 4.75 | 28.73 ± 9.01 | 48.46 ± 3.57 | 35.75 ± 7.37 |
| Perindopril | 10 | 58.69 ± 1.86 | 39.67 ± 2.85 | 39.22 ± 0.97 | 26.81 ± 6.05 |
| TLR4-/-Perindopril | 10 | 51.37 ± 3.71 | 29.99 ± 3.12 | 44.20 ± 2.36 | 32.16 ± 5.48 |

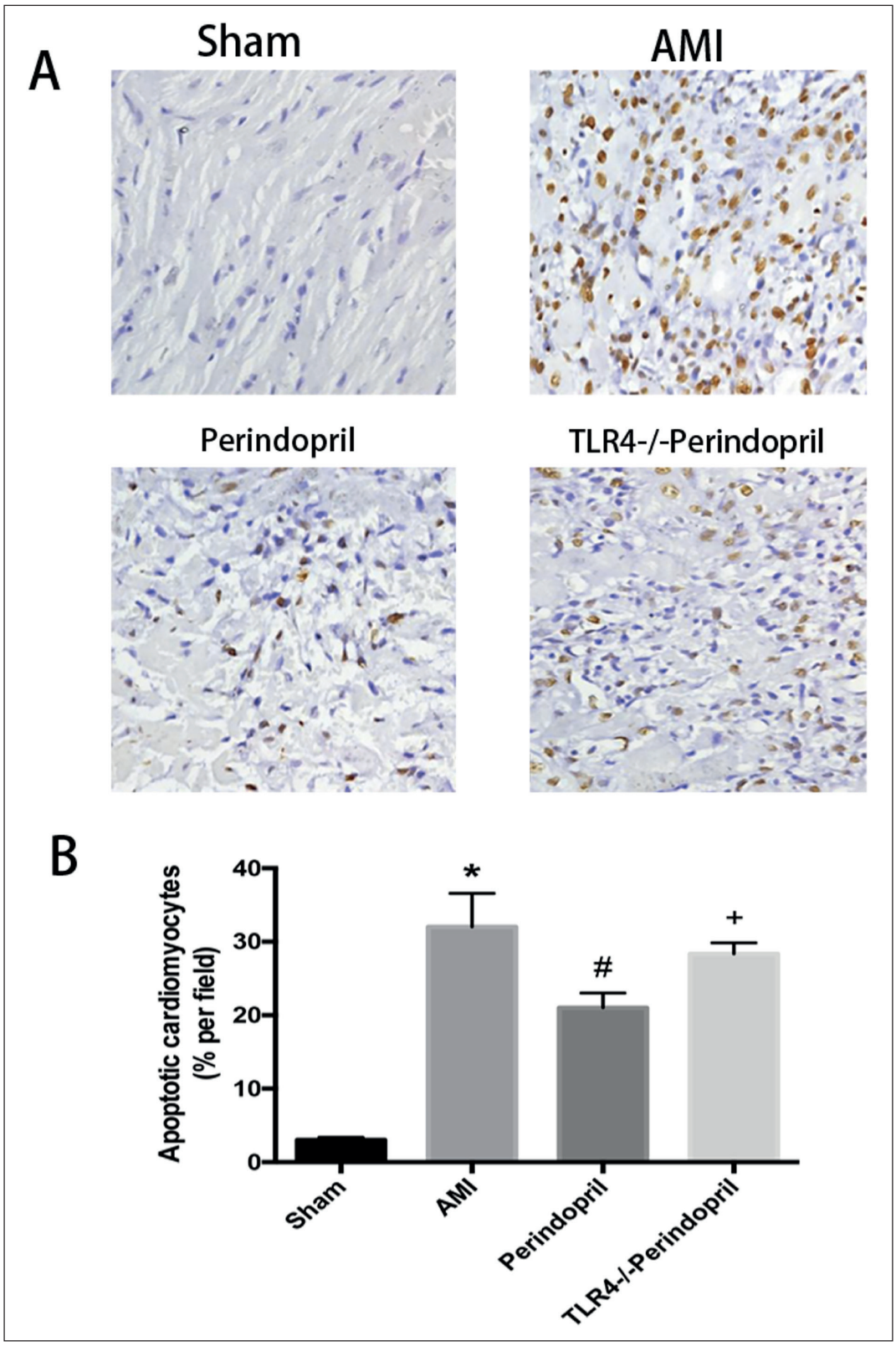


**Figure 1.** ***A,*** TUNEL staining results of myocardial tissues in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group, with brownish-yellow nuclei for positive cells (×400). ***B,*** Quantitative analysis results of apoptosis, expressed as mean ± standard deviation, *:$p<0.05$ compared with the Sham group, #:$p<0.05$ compared with the AMI group, and +:$p<0.05$ compared with the Perindopril group.

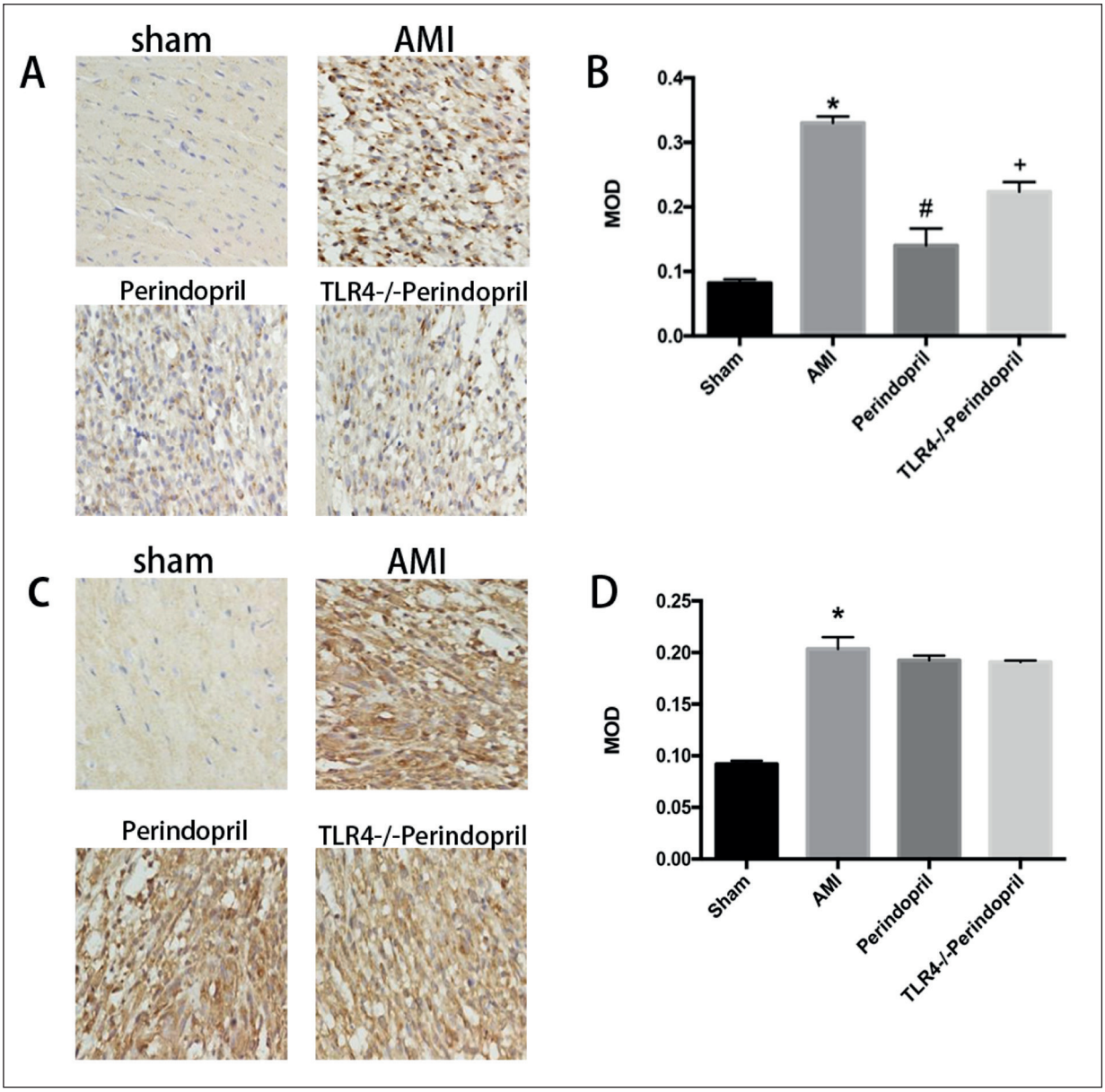


**Figure 2.** ***A,*** Bax protein in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group analyzed *via* immunohistochemistry (400×). ***B,*** Quantitatively-analyzed immunohistochemically mean optical density values, expressed as mean ± standard deviation, *:$p<0.05$ compared with the Sham group, #:$p<0.05$ compared with the AMI group, and +:$p<0.05$ compared with the Perindopril group. ***C,*** Immunohistochemical analysis results of the Bcl-2 protein in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group (400×). ***D,*** Quantitative analysis results of immunohistochemically mean optical density values, expressed as mean ± standard deviation, *:$p<0.05$ compared with the Sham group.

NF-κB p50 protein was expressed in the cytoplasm and nucleus and brown. Compared with the expression in the cytoplasm in the control group, the number of stained NF-κB p50 protein in the nucleus in infarction zones was remarkably increased in the AMI group ($p<0.05$). Compared to the AMI group, the Perindopril group had markedly declined the number of stained NF-κB p50 protein in the nucleus in infarction zones ($p<0.05$). Knockout of TLR4 elevated the reduced number of NF-κB p50 ($p<0.05$; Figure 4B). The results of qPCR and Western blotting showed that the mRNA and protein expression levels of TLR4 in the AMI group were significantly higher than those in the control group ($p<0.05$; Figure 4B, 4D).

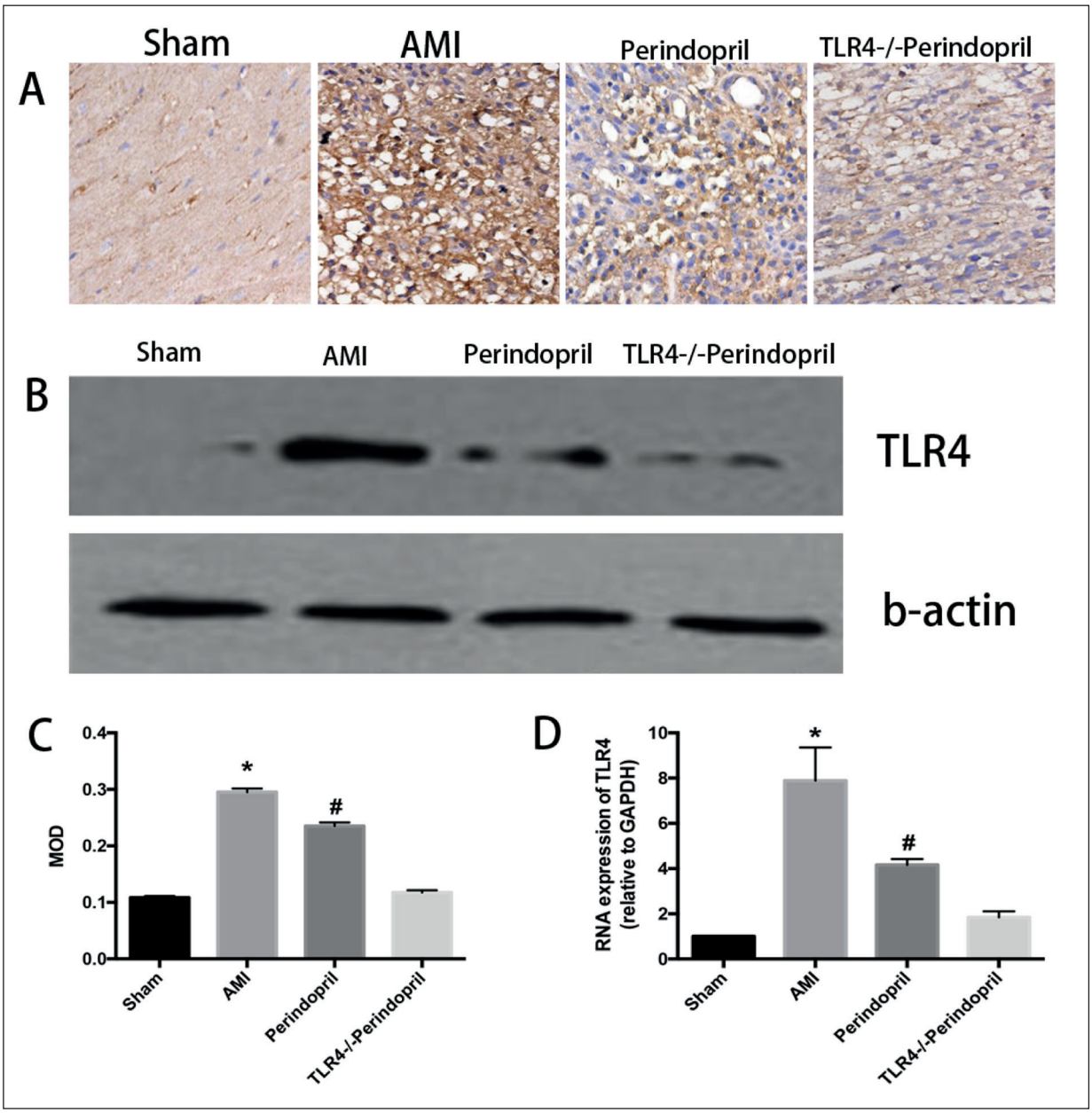


**Figure 3.** ***A,*** TLR4 protein in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group analyzed *via* immunohistochemistry (400×). ***B,*** TLR4 protein content in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group detected through Western blotting. ***C,*** Quantitative analysis results of immunohistochemically mean optical density values, expressed as mean ± standard deviation, *:$p$<0.05 compared with the Sham group, and #:$p$<0.05 compared with the AMI group. ***D,*** TLR4 mRNA content in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group detected *via* Real Time-PCR. *:$p$<0.05 compared with the Sham group, and #:$p$<0.05 compared with the AMI group ($n$=5).

## Discussion

As a mode of active cell death controlled by genes, apoptosis (also known as programmed cell death) plays very important roles in normal growth and development as well as the development of many diseases. Myocardial necrosis and apoptosis are detected after AMI. Moreover, apoptotic myocardial cells are found in the heart of a human with AMI. Veinot et al[5] discovered that myocardial apoptosis appears in infarction zones at 12 h after AMI and is continuously ag-

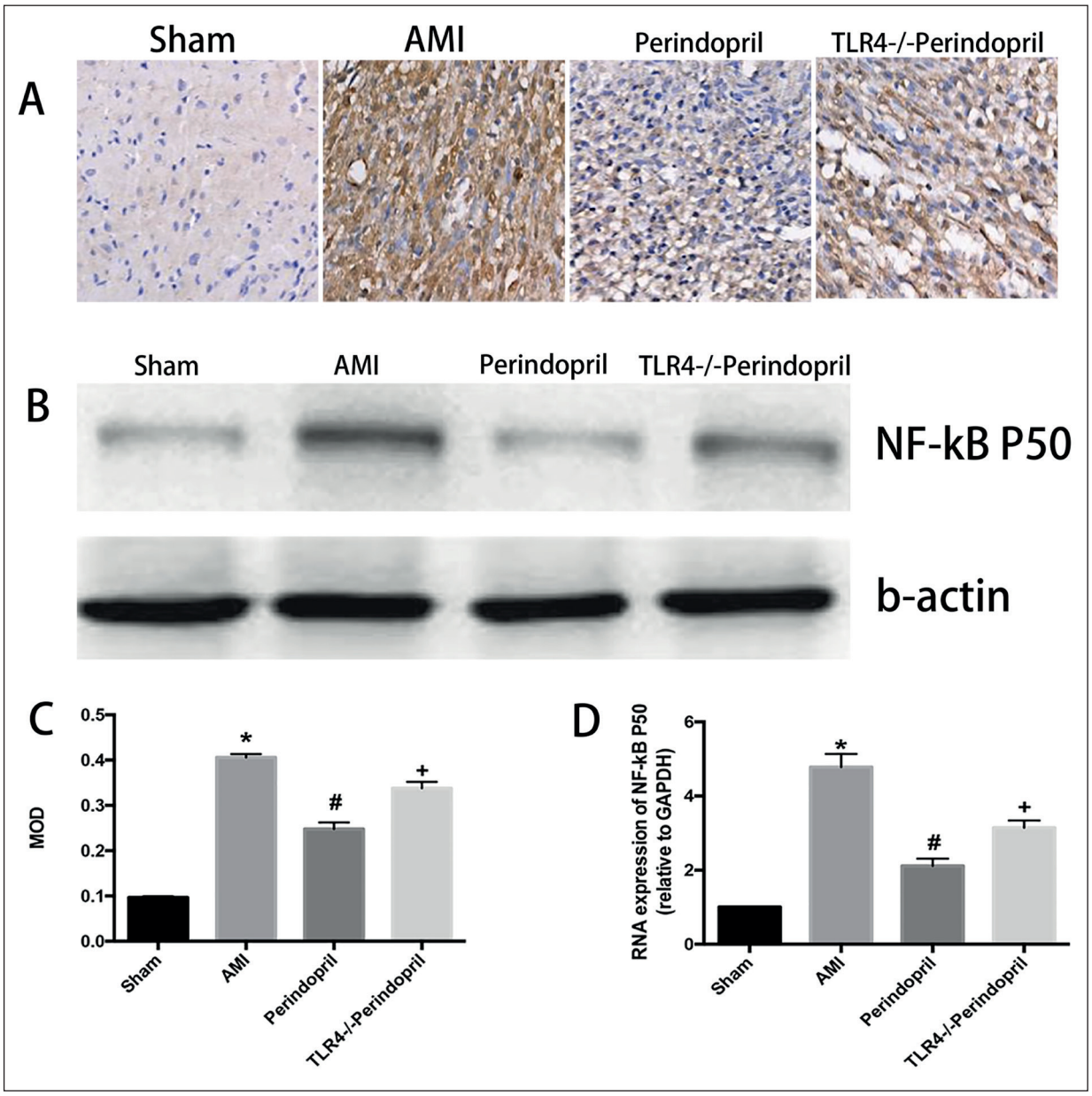


**Figure 4.** ***A,*** NF-κB p50 protein in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group analyzed *via* immunohistochemistry (400×). ***B,*** NF-κB p50 protein content in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group determined through Western blotting. ***C,*** Quantitative analysis results of immunohistochemically mean optical density values, expressed as mean ± standard deviation, *:$p$<0.05 compared with the Sham group, #:$p$<0.05 compared with the AMI group, and +:$p$<0.05 compared with the Perindopril group. ***D,*** NF-κB p50 mRNA content in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group detected *via* Real Time-PCR. *:$p$<0.05 compared with the Sham group, #:$p$<0.05 compared with the AMI group, and +:$p$<0.05 compared with the Perindopril group.

gravated in the next 24-48 h and then gradually relieved. In this work, TUNEL staining revealed that evident myocardial apoptosis was observed in infarction zones at 24 h after establishing mouse models of AMI.

B cell lymphoma/leukemia-2 gene, abbreviated as Bcl-2 gene family, is the first gene that is proved to be capable of repressing apoptosis. Bcl-2 protein inhibits apoptosis due to different factors. Bcl-2 protein expression is found in the excitation and development of many normal cells, but it is not detected or declines in mature or apoptotic cells[14]. Bax protein, another major class of the Bcl protein family, is opposite to

Bcl-2 gene in terms of function, i.e., it promotes apoptosis, which is considered to be an important inducer of mitochondrial damage[15].

At 4-5 h after AMI, Bcl-2 and Bax proteins are scattered in viable myocardial cells around myocardial infarction zones where Bcl-2 and Bax genes are distinctly increased[16]. Other studies have manifested that the development of apoptosis is closely correlated with the expression of Bax (an apoptosis-promoting gene), and depends on the ratio of Bax protein to Bcl-2 protein and the interaction between them, indirectly regulating protease and nuclease activity and regulating apoptosis. The tendency forming homodimers or heterodimers determines the survival or death of cells. When Bax protein is highly expressed, Bax-Bax homodimers are formed, promoting apoptosis. With the increase in the Bcl-2 protein expression level, more and more Bax-Bax dimers are separated and bind to Bcl-2 to form Bax-Bcl-2 heterodimers that are more stable than Bax-Bax. The p53 level regulated by the Bax/Bcl-2 ratio determines the occurrence of apoptosis, so that the apoptosis induced by Bax-Bax homodimers is inhibited[15,17,18]. In recent years, one of the focuses of cell molecular biology studies has been to block apoptosis by interfering in the expression of apoptotic genes to alleviate ischemia-reperfusion injury after AMI. In this work, the protein expression levels of both Bax and Bcl-2 were increased at 24 h after ligating the anterior descending coronary artery of mice.

Studies[19,20] have discovered that the circulatory system and local renin-Ang system are over-activated after myocardial infarction, producing more Ang II that can trigger myocardial apoptosis *via* AT I receptor pathway and thus facilitate myocardial infarction and ventricular remodeling after myocardial infarction. Ang II binds to the AT I receptor and acts on the Bcl-2 family, lowering the Bax/Bcl-2 ratio and inducing apoptosis[21]. Perindopril is a third-generation long-acting ACEI drug capable of effectively blocking the production of Ang II.

In this work, mouse models of AMI were treated with perindopril before establishment for a short term (7 d), TLR4 signaling pathway was activated and the influences of perindopril on myocardial apoptosis, Bax, Bcl-2 and TLR4-NF-κB signaling pathway were observed. It was found that the expression levels of Bax, Bcl-2, TLR4 and NF-κB in myocardial tissues in myocardial infarction zones in mouse models of AMI were elevated, and there were many apoptotic myocardial cells. Perindopril was applied to intervene in models, and the results revealed that perindopril inhibited the expression of TLR4 protein and decreased the nucleus transfer level of NF-κB, the expression of Bax in myocardial cells in myocardial infarction zones was decreased, and the number of apoptotic myocardial cells declined. We found that perindopril suppressed the TLR4 protein expression, but the relevant mechanism remains unclear. Whether perindopril directly inhibits the expression of TLR4 protein or represses it by blocking the expression of NF-κB protein has not been proved, so further studies are needed.

This work demonstrates that prophylactic application of perindopril can reduce the protein expressions of TLR4 and NF-κB to relieve the apoptosis of myocardial cells in infarction zones in models of AMI and lower the ratio of Bax (a pro-apoptotic protein) to Bcl-2 (an anti-apoptotic protein).

## Conclusions

We demonstrated that perindopril inhibits myocardial apoptosis in mice with AMI through the TLR4/NF-κB pathway.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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