European Review for Medical and Pharmacological Sciences 2019; 23: 6672-6682

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

X.-Z. WANG[1], Z.-X. YU[1], B. NIE[2], D.-M. CHEN[3]

[1]Department of Ultrasonography, Dongzhimen Hospital of Beijing University of Chinese Medicine, Beijing, China
[2]Key Laboratory, Dongzhimen Hospital of Beijing University of Chinese Medicine, Beijing, China
[3]Pharmacological Research, Dongzhimen Hospital of Beijing University of Chinese Medicine, Beijing, China

Xiangzhu Wang and *Zexing Yu* contributed equally to this work.

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

MATERIALS AND MATERIALS: A total of 48 mice were randomly divided into 4 groups before intervention, namely sham operation (Sham group, n=12), AMI group (n=12), 1 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 days. On the 7th day after drug administration, mice in the AMI group, Perindopril group and TLR4-/-Perindopril group were subjected to ligation of the anterior descending coronary artery to induce AMI, and those in the Sham group underwent the same operation without a ligature knot at the anterior descending coronary artery. At 24 h after the above operation, color echocardiography was performed on the 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 AMI group. AMI mice in the Perindopril group showed 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

Corresponding Author: Xiangzhu Wang, MM; e-mail: wxzhyzx@126.com

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 k light-chain-enhancer of activated B cell κB)[6]. NF-κB is a widely-expressed transc factor affecting immune regulation, cell m tion and apoptosis. An experiment proved t TLR4 messenger ribonucleic a RNA) e pression is detected in myoca ce neona- tal rats, which is increased f stimu on with lipopolysaccharide (LPS terleuk 1β) and Interferon-γ (N- ng N and declines when κB is d by PDTC (a specific NF-κB ker)[7]. The l lympho- ma 2 (Bcl-2) breviation e B cell lymphoma/leu mia-2 is the first gene that is confirm o be able to it apoptosis. Bcl-2 protein suppress apoptos duced by differ- ent f s. Ma hita et al[8] found that activated NF s can of mediating apoptosis through Bcl-2. ve exp ents have fully proven the 4 sig g pathway is associated w myoca ptosis, and apoptosis can be rep ed by king different signaling mol- ec the TLR4 signaling pathway through ods.

Recent experimental studies[9,10] have manifested that the application of certain medications be 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 s and the inhibition of angiotensin II can reduce the activity of NF-κB addition, ACEI drugs can suppress NF-κB ty in myocardial cells in rats with myocardi rction[13]. This experiment aims to o ve th ct of perindopril pretreatment xperiment and myocardial apopto mice.

Mate s an thod

Animals a gents

A total 6 C 6J mice aged 8 weeks and weighing (18.2 ± 0. nd 12 TLR4 knockout mic weeks and ing (20.4 ± 0.4) kg, b with normal nutritional status and mental s s, were pro ed by the Laboratory Animal er of Beiji University of Chinese Medi This stud as approved by the Animal Eth mm of Beijing University of Chinese M Animal Center. Bcl-2-associated X protein (Bax), Bcl-2, TLR4, NF-κB p50 antiwere purchased from Sigma-Aldrich (St. ou O, 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 ... perature constant. Then, an appropriate ... of ultrasonic coupling agent was applied ... precordial region; the 30 MHz probe of the u... sound system was used to collect B-Mode ima... of the parasternal long axis vie... pical fo... chamber view and record the ... iogram. The M-shaped section im... of the ... asternal left ventricular short a... s ob... level of chordae te... ea... valve. ... ventricular end-sys... diame... VESD), left ventricular end-... lic period ... DD), left ventricular dia... erior wall th... ess, left ventricular di... olic ve... and left ventricular ejection f... ion (LVEF) ... e measured. For differe... dividuals, the pa... eter settings of the V... 700 w... ound system were the same. Fo... me... ement index, three consecutive cardia... were se... d, and the average was ... n. A... hoca... graphy, mice were killed, t... est w... d, and the heart was taken out... hen, the ... idual blood was washed off wi... Phosphate-Buffered Saline (PBS; Gib... St... nd, NY, USA), followed by drying ... th 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_2O_2 (Sigma-Aldrich, St. Louis, MO, USA) for 30 min. After the endogenous peroxide was eliminated, the sections were blocked with serum and ... with primary antibodies for incubati... overnight. On the next day, the s... ons were incubated with immunoglobuli... (IgG) antibody-horseradish peroxidase (... Abcam, Cambridge, MA, USA) a... dropw... dded with the mixture prepared ... e biotin a... kit (TaKaRa, Otsu, Shi... apan) f... culture... lowed by color devel... nt wit... aminobenzidine (DAB; Solarb... ... na) fo... min. Thereafter, sect... wer... nterst... d with hematoxylin. ... ned, dehy... ... permeabilized. La... ections wer... erved using an optica... cros...

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

...raffin-embe... d tissue sections were dep... ...ized with ... ene, dehydrated with graded alc... ... d with Proteinase K working solutio... 30 min at 21-37°C or added with ... cell-permeable solution for 8 min of reaction. ... (Roche, Basel, Switzerland) reaction ...ix... 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 to 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 (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 Taq II (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 cDNA solution and 6 µL of sterile ddH$_2$O. PCR parameters: pre-denaturation at 95°C for 30 s, 95°C for 5 s, at 60°C for 34 s, fluorescence collection at 60°C for 40 cycles. Data were collected using the Applied Biosystems 7500 Fast Real-time PCR System (Foster City, CA, USA). GAPDH as housekeeping gene, the relative expression level was calculated by the $2^{-\Delta\Delta Ct}$ method. The following primers were used to detect Bcl-2 (forward primer 5′-AAGGCATGGCATCGCTTACAC-3′, reverse primer 5′-TGTTCTCCACAGCCACCAGAT-GTC-3′), NF-κB P50 (forward primer 5′-GACTACACAGAACTCAGCATCC-3′, reverse primer 5′-CCAAGCAGCAGCAGACATG-3′), and GAPDH (forward primer 5′-GCCTCGTCCCGTAGACAAAA-3′, reverse primer 5′-GATGGTCCCGTTGATGA-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 lo[wered] the number of apoptotic myocardial cells [in] parison with the AMI group ($p<0.05$), whil[e] a decrease was counteracted by TLR4 knoc[k] ($p<0.05$; Figure 1).

Perindopril Reduced the [Expr]ession Levels of Myocardial B[ax] and B[cl]-2 in Infarction Zones of M[ice] With[out] AMI

Immunohistochemi[stry] w[as] [us]ed to [mea]sure the protein e[xp]ression l[evel]s of Bax and Bcl-2. The cells [were] [stai]ned for stainin[g] brown or brownish-yello[w] [and] localized in the [cy]toplasm. Image-Pro Plu[s] 6.0 ([Versio]n X; Media Cybernetics, Si[lver] Springs, M[D], USA) was utilized for calc[ulat]ion of the averag[e] optical density in each [gro]up and [pro]tein quantification. The more the [brow]nish-yellow and the darker the color was, the greate[r] [the] average optical density value would [be]. We f[ound] 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 tre[atment] (Figure 2A, 2B). Bcl-2 protein is also [mainly] in the cytoplasm. Bcl-2 protein ex[pres]sion was observed in each group ($p<0.0$[5], which was overtly elevated in the AMI gro[up] and Perindopril group compared with [that] in [the] control group ($p<0.05$). There wa[s] significant[] ence in Bcl-2 protein [e]xpression between th[e] AMI g[roup] and Perindopril grou[p] (Figure 2[C], 2D). However, the protein expressi[on] [of] Bax and Bcl-2 in TLR4-/-Perindo[pril] group [was] incre[ased] compared with th[at] in the Per[indo]pril group. The above resul[ts] [sho]w that the trea[tment] with perindopril ca[n] reduce [the] Bax gene and protein expression levels of Bax (a my[ocar]dial apoptosis gene) in infa[rction] zones in mic[e] with AMI, and TLR4 k[nock]out can attenuate thi[s] effect of perindopril [to] some extent.

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

The protein expression of TLR4 in infarction [zones] was detected *via* immunohistochemistry. [TLR4] 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).

T[able] [I] [Com]parison 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

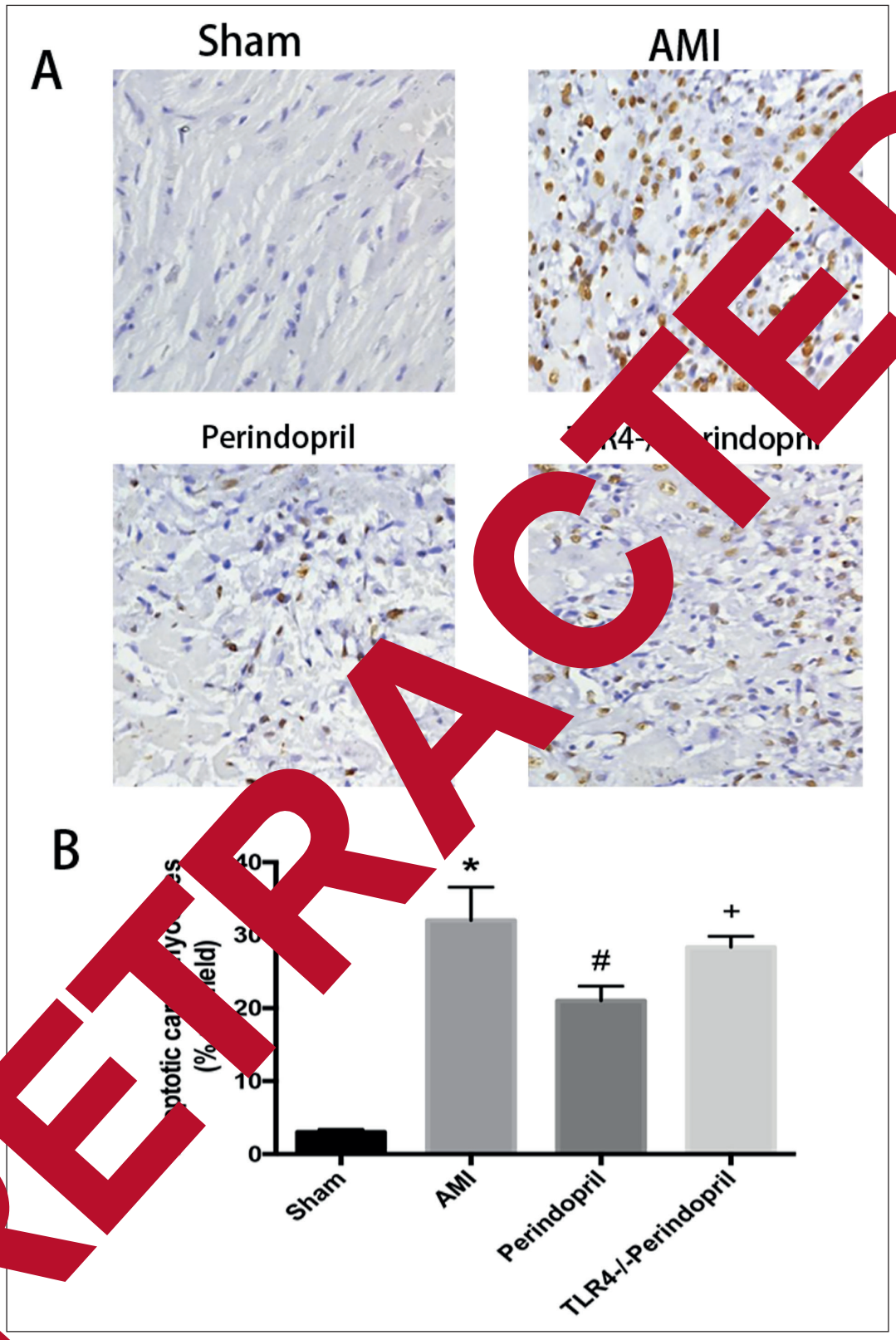


Figure 1. ***A,*** TUNEL staining results of myocardial tissues in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group, with brownish-yellow nuclei for positive cells (×400). ***B,*** Quantitative analysis results of apoptosis, expressed as mean ± standard deviation,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

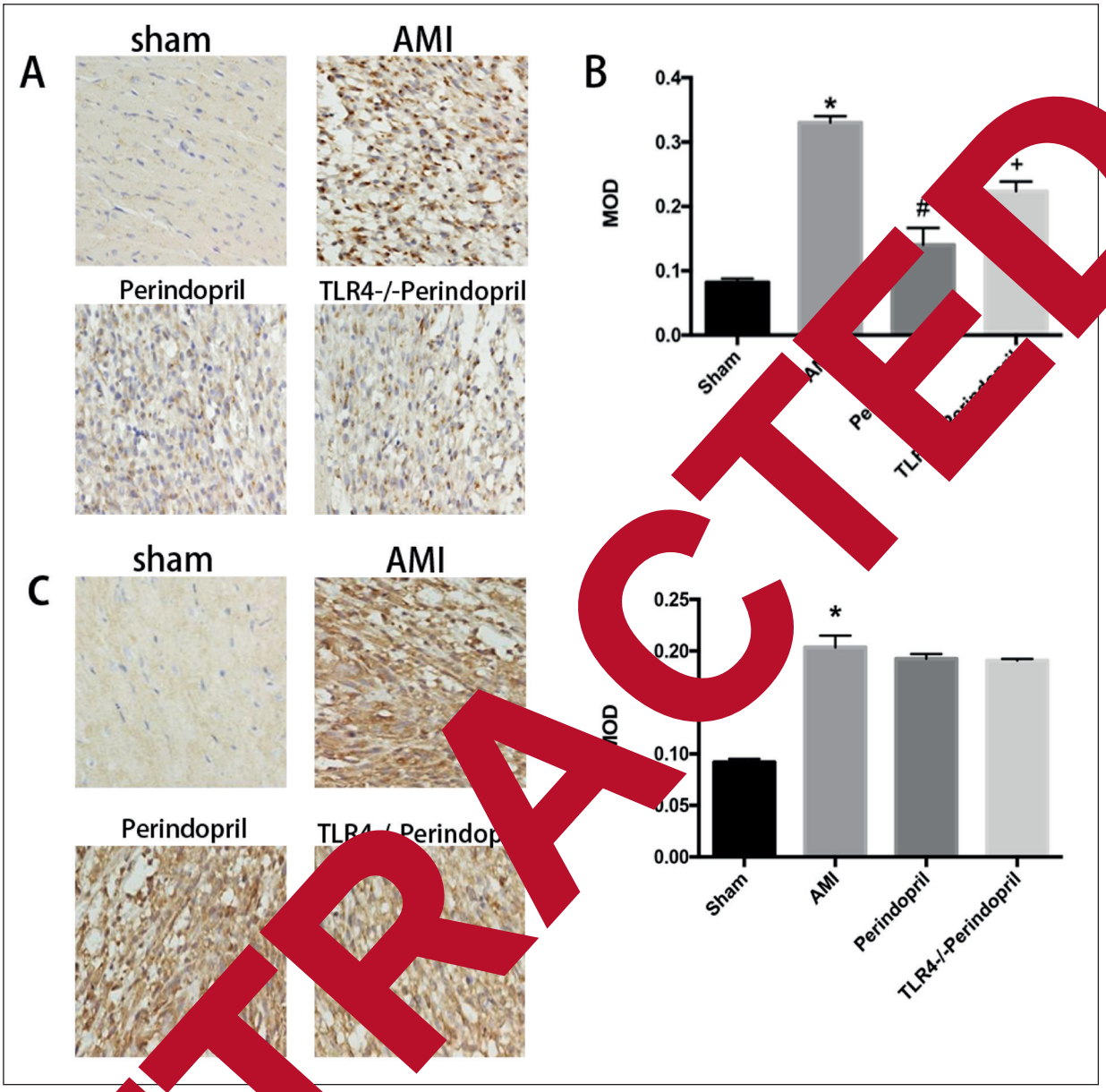
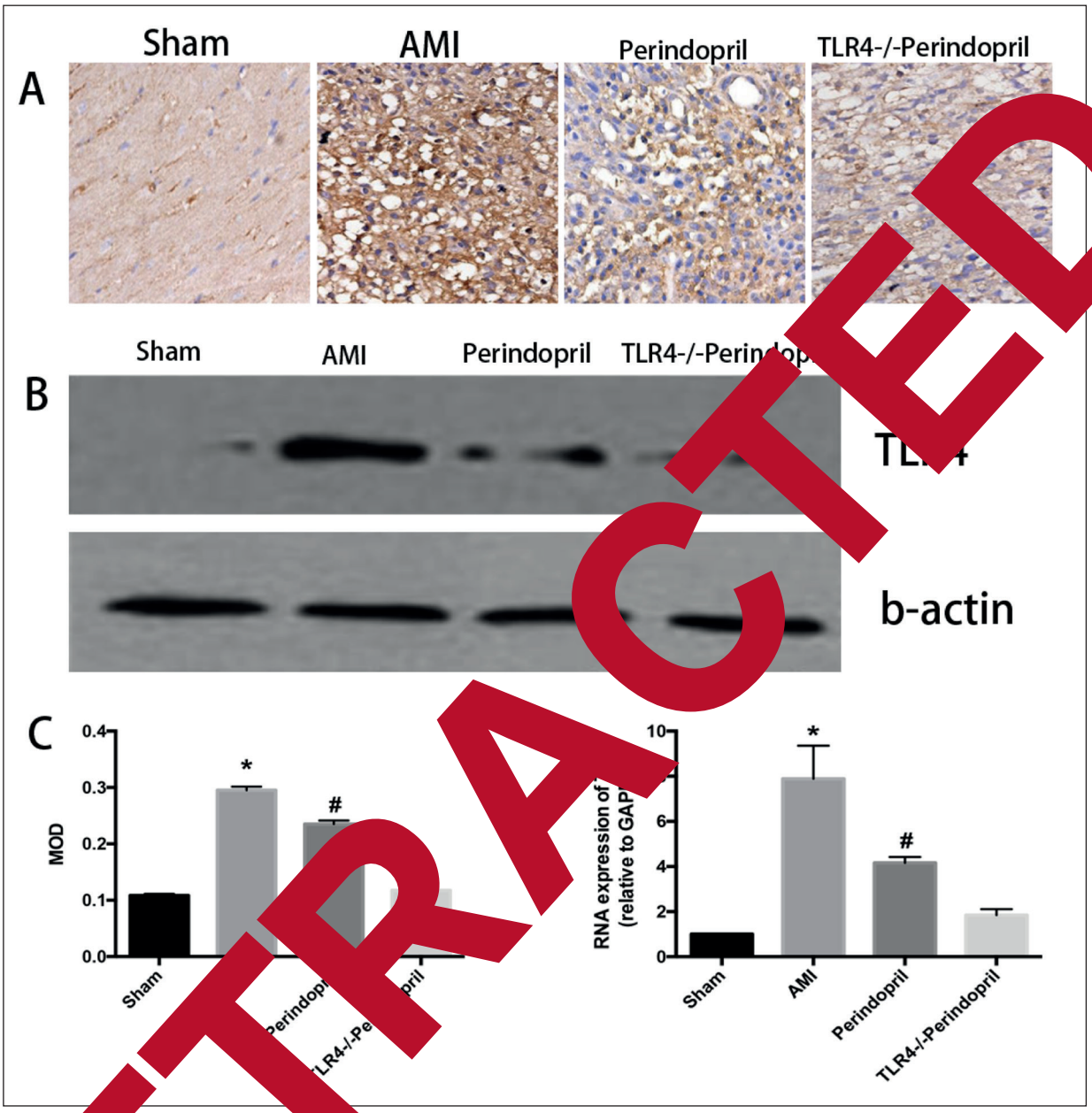


Figure 2. ax protein in in zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group analyze mmunohistochemisti 0×). ***B,*** Quantitatively-analyzed immunohistochemically mean optical density values, expre s mean andard deviation, *:$p<0.05$ compared with the Sham group, #:$p<0.05$ compared with the AMI group, an .05 co d with the Perindopril group. ***C,*** Immunohistochemical analysis results of the Bcl-2 protein in infarction zones group, A roup, Perindopril group and TLR4-/-Perindopril group (400×). ***D,*** Quantitative analysis results of immu hemical an optical density values, expressed as mean ± standard deviation, *:$p<0.05$ compared with the group

NF- 50 protein was expressed in the cyto- n cleus and brown. Compared with e expression in the cytoplasm in the control p, the number of stained NF-κB p50 protein i 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).



Figur ***A,*** TLR protein in infar ion zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril grou lyzed mmunohistochemistry (400×). ***B,*** TLR4 protein content in infarction zones in the Sham group, AMI grou ndo roup an TLR4-/-Perindopril group detected through Western blotting. ***C,*** Quantitative analysis results of immun ically m ptical density values, expressed as mean ± standard deviation, *:p<0.05 compared with the Sham , and 05 co d with the AMI group. ***D,*** TLR4 mRNA content in infarction zones in the Sham group, AMI group, pril g K4-/-Perindopril group detected *via* Real Time-PCR. *:p<0.05 compared with the Sham group, and #:p compa the AMI group (n=5).

Discussion

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-

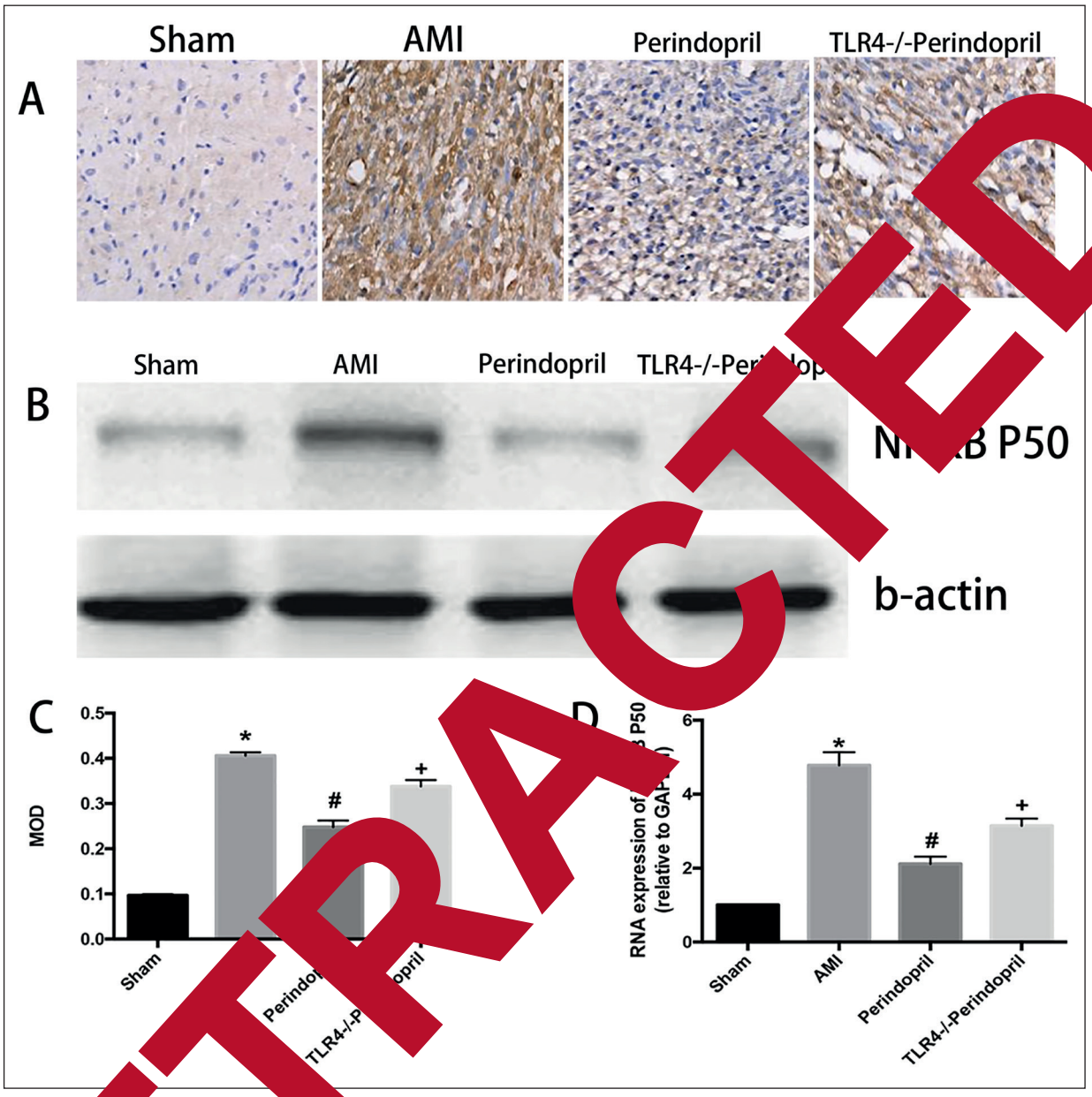


Figure 4. NF-κB p50 protein in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group analyzed by immunohistochemistry (400×). ***B,*** NF-κB p50 protein content in infarction zones in the Sham group, AMI group, Perindopril group and TLR4-/-Perindopril group determined through Western blotting. ***C,*** Quantitative analysis results of immunohistochemically measured data 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.

...e next 24-48 h and then gradually ...ieved. In this work, TUNEL staining revealed ...evident myocardial apoptosis was observed in ...arction 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 f es of cell molecular biology studies has block apoptosis by interfering in the expr of apoptotic genes to alleviate ischemia-rep sion injury after AMI. In this work, the prot expression levels of both Bax cl-2 we increased at 24 h after ligat rior de- scending coronary artery ce.

Studies[19,20] have disco that th system and local reni Ang re ove tivated after myo al infa producing more Ang II that trigger myo al apopto- sis *via* AT I re way and t acilitate myocardial in ction a ntricular remodeling after myo ial infarctio g II binds to the AT I re or and acts on th -2 family, low- erin Bax/ 2 ratio and inducing apopto- sis indo s a third-generation long-acting ACE pable fectively blocking the luct Ang

his use models of AMI were trea with pe opril before establishment for a m (7 d), TLR4 signaling pathway was the influences of perindopril on yocardial apoptosis, Bax, Bcl-2 and TLR4- κB signaling pathway were observed. It was fo 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 l of NF-κB, the expression of Bax in myoc in myocardial infarction zones w ecreased, and the number of apoptotic m rdial cells declined. We found that perindop pressed the TLR4 protein expressi but t vant mechanism remains uncle hether per directly inhibits the ex ion of LR4 pr or represses it by blo the e ssion of NF- κB protein has not ee further dies are needed.

This work onstrates t actic application of pril can re the protein expressio f T nd NF-κB to relieve the apoptosis of myocar lls in infarction zones in n f AMI and the ratio of Bax (a p poptotic protein) to Bcl-2 (an anti-apoptotic ein).

onclusions

We demonstrated that perindopril inhibits rdial apoptosis in mice with AMI through e 4/NF-κB pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Kivimaki M, Nyberg ST, Batty GD, Fransson EI, Heikkila K, Alfredsson L, Bjorner JB, Borritz M, Burr H, Casini A, Clays E, De Bacquer D, Dragano N, Ferrie JE, Geuskens GA, Goldberg M, Hamer M, Hooftman WE, Houtman IL, Joensuu M, Jokela M, Kittel F, Knutsson A, Koskenvuo M, Koskinen A, Kouvonen A, Kumari M, Madsen IE, Marmot MG, Nielsen ML, Nordin M, Oksanen T, Pentti J, Rugulies R, Salo P, Siegrist J, Singh-Manoux A, Suominen SB, Vaananen A, Vahtera J, Virtanen M, Westerholm PJ, Westerlund H, Zins M, Steptoe A, Theorell T. Job strain as a risk factor for coronary heart disease: a collaborative meta-analysis of individual participant data. Lancet 2012; 380: 1491-1497.

2) Zhou M, Zou YG, Xue YZ, Wang XH, Gao H, Dong HW, Zhang Q. Long non-coding RNA H19 protects acute myocardial infarction through activating autophagy in mice. Eur Rev Med Pharmacol Sci 2018; 22: 5647-5651.

3) Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa

P. Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. Lab Invest 1996; 74: 86-107.

4) Akasaka Y, Morimoto N, Ishikawa Y, Fujita K, Ito K, Kimura-Matsumoto M, Ishiguro S, Morita H, Kobayashi Y, Ishii T. Myocardial apoptosis associated with the expression of proinflammatory cytokines during the course of myocardial infarction. Mod Pathol 2006; 19: 588-598.

5) Veinot JP, Gattinger DA, Fliss H. Early apoptosis in human myocardial infarcts. Hum Pathol 1997; 28: 485-492.

6) Yan ZQ. Regulation of TLR4 expression is a tale about tail. Arterioscler Thromb Vasc Biol 2006; 26: 2582-2584.

7) Frantz S, Kobzik L, Kim YD, Fukazawa R, Medzhitov R, Lee RT, Kelly RA. Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. J Clin Invest 1999; 104: 271-280.

8) Matsushita H, Morishita R, Nata T, Aoki M, Nakagami H, Taniyama Y, Yamamoto K, Higaki J, Yasufumi K, Ogihara T. Hypoxia-induced endothelial apoptosis through nuclear factor-kappaB (NF-kappaB)-mediated bcl-2 suppression: in vivo evidence of the importance of NF-kappaB in endothelial cell regulation. Circ Res 2000; 86: 974-981.

9) Reinhardt D, Sigusch HH, Hensse J, Tyagi SC, Körfer R, Figulla HR. Cardiac remodelli in end stage heart failure: upregulation of metalloproteinase (MMP) irrespective of derlying disease, and evidence for a dir hibitory effect of ACE inhibitors on MMP. 2002; 88: 525.

10) Wilson WR, Evans J, Bell PR, T MM. HM CoA reductase inhibitors (st se MMP 3 and MMP-9 concentrati n abd al aortic aneurysms. Eur J Vasc ovasc S 005; 30: 259-262.

11) Cigola E, Kajstura , M nversa P. Angiotensin II acti s progra myocyte cell death in vitro ell Res 199 363-371.

12) Douillette er A, Gravel lement JF, Chenard V, oreau ant MJ. The proinflammatory ions of angi II are dependent on p65 phosphorylation by the IkappaB kinase complex. J Biol Chem 2006; 281: 13275-13284.

13) Yoshiyama M, Omura T, Takeuchi K, Kim S, Shimada K, Yamagishi H, Teragaki M, Akioka K, Iwao H, Yoshikawa J. Angiotensin blockade inhibits increas AP-1 and NF- kappa B DNA-bindin myocardial infarcted rats. J Mol Ce diol 2001; 33: 799-810.

14) Tsujimoto Y, Cossman J, Jaffe E, Cr Involvement of the bcl-2 gene in h an follic mpho-ma. Science 1985; 228: 1443.

15) Oltvai ZN, Milliman C orsmeyer SJ. Bcl erodimerizes in viv a co ed homo , Bax, that accele rogr ed cell death. Cell 1993; 74: 6 -6

16) Misao J, Ha Y, Oh Kato iwara T, Fujiwara H ression of b an inhibitor of ap nd Bax, an ator of apoptosis, ent myocytes human hearts with m cardial ion. Circulation 1996; 94: 1 12.

17) M, Barrans JD, KS, Liew CC. Apoptosis-related genes expressed in cardiovascular developm and disease: an EST approach. Cardiovasc F 000; 45: 621-629.

18) vakumaran n HK, Sjin RT, Reed JC, Liebermann Hoffm The novel primary response gene M the proto-oncogenes myb, myc, and bcl-2 modulate transforming growth factor beta 1-induced apoptosis of myeloid leukemia cells. Cell Biol 1994; 14: 2352-2360.

19) K GT, Chun YS, Park JW, Kim MS. Role of apoptosis-inducing factor in myocardial cell death by ischemia-reperfusion. Biochem Biophys Res Commun 2003; 309: 619-624.

20) Zhu BQ, Sievers RE, Browne AE, Lee RJ, Chatterjee K, Grossman W, Karliner JS, Parmley WW. Comparative effects of aspirin with ACE inhibitor or angiotensin receptor blocker on myocardial infarction and vascular function. J Renin Angiotensin Aldosterone Syst 2003; 4: 31-37.

21) Holtz J. Role of ACE inhibition or AT1 blockade in the remodeling following myocardial infarction. Basic Res Cardiol 1998; 93: 92-100.