Renal Denervation Ameliorates Cardiomyocyte Apoptosis in Myocardial Ischemia-reperfusion Injury

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Research Article

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Abstract

Background: Myocardial ischemia-reperfusion injury (I/R) has been improved with drugs and effective reperfusion, but it still cannot be prevented. We compared the effects of renal denervation (RDN) and angiotensin receptor neprilysin inhibitors (ARNIs) on cardiomyocyte apoptosis after I/R to explore whether RDN can reduce cardiomyocyte apoptosis by improving endoplasmic reticulum stress.

Methods: Sixty male specific pathogen free (SPF) Wister rats were randomly divided into six groups. (n=6):(a) normal; (b) sham; (c) I/R; (d) RDN; (e) I/R+RDN and (f) I/R+ARNI. We established the I/R rat model by ligating the left anterior descending artery. Rats were exposed tThe renal artery was exposed and then smeared phenol on the vessel for chemical ablation. The I/R+ARNI group received ARNIs for 2 weeks until killed. We also collected the cardiac tissues and blood serum to determine I/R-related indicators and analyze the potential mechanisms involved.

Results: The levels of Norepinephrine (NE), Ang II, and aldosterone (ALD)increased significantly in the I/R group but decreased significantly after RDN and ARNI intervention. The expression of Bax, caspase-3, CHOP, PERK, and ATF4 protein was significantly increased in the I/R group, which compared to other groups, and the level of CHOP, PERK, and ATF4 gene expression increased. After RDN intervention, these expression levels recovered to varying degrees.

Conclusion: Our results provide new evidence that RDN ameliorates cardiomyocyte apoptosis in I/R. The effect of RDN may be associated with regulating the endoplasmic reticulum stress PERK/ATF4 signaling pathway.

Introduction

Acute myocardial infarction (AMI) remains a leading cause of mortality and morbidity worldwide [1]. AMI leads to many kinds of complications, including recurrent myocardial infarction, sudden cardiac death, heart failure, and stroke [2–3]. Primary percutaneous coronary intervention and thrombolytic therapy are still the main interventions for AMI to restore early recovery of myocardial blood flow [4]. Although timely revascularization can reduce myocardial infarct size and improve cardiac function, it causes secondary damage to the myocardium and contributes to further cardiomyocyte necrosis and apoptosis, which is termed myocardial ischemia-reperfusion (I/R) injury [5]. The occurrence of I/R can activate the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS), which play essential roles in the pathogenesis of cardiovascular disease, especially acute myocardial infarction. Hyperactivation of the RAAS and sympathetic overactivity are thought to trigger cardiomyocyte apoptosis [6]. Therefore, in order to attenuate cardiomyocyte apoptosis, the suppression of excessive activation of the SNS and RAAS has become a hot topic in clinical studies.

As a new treatment option, renal denervation (RDN) has been used in the treatment of refractory hypertension, chronic heart failure, and atrial fibrillation [7–9]. RDN can block renal transmission nerves, specifically and effectively reducing the activity of the SNS and RAAS, improving cardiac function and

ventricular remodeling [10]. Currently, we know that norepinephrine and Ang II play important roles in myocardial apoptosis, and Wang et al [11] found that RDN can reduce the aldosterone and Ang II concentrations in a model of heart failure. Wang et al [12] also confirmed that RDN attenuates cardiac fibrosis.

Many drugs have been applied in I/R injury, such as angiotensin-converting enzyme inhibitor(ACEI) and angiotensin II receptor blocker (ARB), but the overall treatment is not satisfactory. A novel drug class, angiotensin receptor neprilysin inhibitors (ARNIs), has been used in clinical therapy, and some studies have confirmed that they are the best clinical choice for attenuating cardiac fibrosis [13–15].

Cardiomyocyte apoptosis plays an important role in the development of ventricular remodeling and heart failure after myocardial infarction. Endoplasmic reticulum stress (ERS) is another important apoptosis pathway that participates in cardiomyocyte apoptosis following mitochondrial and death receptor pathways [16]. Previous studies have shown that myocardial ischemia can induce ERS [17], which can activate apoptotic signaling, such as C/EBP-homologous protein (CHOP), and cause apoptosis, promoting the occurrence of myocardial disease [18–20]. Three proximal transmembrane signal transduction molecules are involved in ERS signal transduction: protein kinase RNA-like ER kinase (PERK), inositol-requiring protein-1 (IRE-1), and activating transcription factor 6 (ATF6). PERK promotes phosphorylation of translation initiation factor eIF2α and induces translation of activating transcription factor 4 (ATF4), which is involved in the proapoptotic process [21–23]. ATF4 can also induce the expression of CHOP [21].

Therefore, we designed this study to investigate whether RDN and ARNIs can reduce cardiomyocyte apoptosis, and we sought to explore whether RDN can reduce apoptosis in rats with I/R injury via the ERS-associated PERK/ATF4 signaling pathway.

Materials And Methods

Experimental animals and treatment.

Sixty healthy adult male specific pathogen free (SPF) Wister rats (7 weeks old) weighing 200–250 g were purchased from Hubei Province Laboratorial Animal Center (Hubei, China). All the experiments were conducted in accordance with the guide for the Care and the Use of Laboratory Animials and were approved by the Laboratory Animal Ethics Committee of Tianjin First Centre Hospital (Tianjin, People's Republic of China [PRC]). The quality of included studies was assessed by using Animal Research:Reporting in Vivo Experiments(ARRIVE) guidelines. The rats were housed in the departmental animal house and kept under controlled lighting conditions (light: dark, 12 h: 12 h) with an ambient temperature of 22 ± 2 °C, relative humidity of 40%-60%, and free access to food and water. All of the rats were maintained for 7 days prior to the experimental procedures and then randomly assigned to six groups (n = 10 rats per group): the *normal* group, rats in this group did not undergo any kind of processing; the *sham operation* group, rats in this group underwent surgical manipulation, exposure of

the renal artery without ligating the left anterior descending coronary artery (LAD), and treatment of the vessel with 0.9% saline; the *I/R* group, the LAD was ligated as described below and reperfused for 1 week, then laparotomy was performed to expose the renal artery, which was treated with 0.9% saline; the *RDN* group, rats in this group underwent surgical manipulation without ligating the LAD, then laparotomy was performed to expose the renal artery and phenol applied to the vessel for chemical ablation; the *I/R + RDN* group, the rats in this group underwent surgical manipulation with ligation of the LAD and reperfusion, then 1 week later laparotomy was performed to expose the renal artery and phenol applied to the vessel for chemical ablation; the *I/R + ARNI* group, the rats in this group underwent surgical manipulation with ligation of the LAD and reperfusion, and then administered oral ARNIs (60 mg/kg/day) for 2 weeks until killed. The doses used in the experiment were based on a previous study [24]. We found no significant differences in breed, body weight, age, or sex among the six groups. All rats were sacrifificed using an i.p. injection of an overdose of pentobarbital sodium.

I/R injury procedures.

The I/R model was established similar to a previous study [25]. Briefly, the rats were anesthetized using an R540 series anesthetic machine (RWD Life Science, Shenzhen, China) and fixed in a supine position for endotracheal intubation using a small animal respirator at the rate of 60 breaths/min and 1:1 suction ratio (R415, RWD, China). After removing the hair, a small incision was made in the left thoracic cavity. We opened the chest carefully and exposed the heart. The LAD was ligated using 6 – 0 silk sutures with a section of PE-10 tubing placed over the LAD for 30 min. The myocardium turned white, and their condition was confirmed by ST segment elevation in lead II of the electrocardiogram (ECG; Fig. 1). After the myocardial ischemia appeared to be successful, we released the ligature and closed the chest. The rats were placed in a cage with clean bedding, and all animals were given penicillin (80,000 u) for 3 days.

RDN procedures.

One week after ligation surgery, RDN and sham surgery were performed as described previously [26]. First, we used the R540 series anesthetic machine (RWD Life Science, Shenzhen, China), intubated, and ventilated using a rodent ventilator. Next, we exposed both kidneys. After isolating the surrounding connective tissue and periadventitial fat, we identified the renal arteries and veins. All visible nerves were severed bilaterally. We carefully painted the renal vessels vessel with phenol (10% phenol in 95% ethanol) using a cotton swab for 2 min to destroy the remaining nerves. The rats in the sham group were painted with 0.9% saline without destruction of the bilateral renal nerves.

ARNI procedures.

One week after reperfusion, the rats in one subgroup were fed ARNIs (60 mg/kg) for 2 weeks and then sacrificed. The hearts and serum were collected and preserved at -80 °C.

Echocardiography.

Two-dimensional echocardiography (DP-50ev, mindray, China) was performed 1 week after I/R (baseline level) and 2 weeks after RDN or ARNI treatment (3 weeks after I/R). Left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVSD), left ventricular ejection fraction (LVEF), and left ventricular fractional shortening (LVFS) were chosen for analysis. The heart rate (HR) and blood pressure were monitored at the same time. A four channel physical recorder (BL-420F systems, Chengdu Technology and Market, China) was used to monitor the ECG signals.

Enzyme immunoassay of NE, Ang II, and ALD.

After 3 weeks of reperfusion, blood samples were collected and centrifuged for 30 min at 3000 *g*. Myocardial tissue was also collected and centrifuged for 10 min at 5000 *g*. The supernatant was stored at -80 °C until enzyme-linked immunosorbent assay (ELISA). The levels of Norepinephrine (NE), Ang II, and aldosterone (ALD) were determined using commercial ELISA kits (Wuhan Elabscience Biotechnology Co., Ltd, Wuhan, China) following the manufacturer's guidelines.

2,3,5-Triphenyltetrazolium chloride (TTC) staining.

At the end of the reperfusion, the rats were sacrificed immediately for TTC staining. The hearts were removed and washed with physiological saline solution. The myocardial tissues were frozen at -20 °C for 20 min and sliced into 2-mm-thick sections. After incubation with 1% TTC solution (Beyotime, Shanghai, China) at 37 °C for 15 min, each section was photographed. The infarct area was stained white, and the non-infarct area was stained red.

Hematoxylin-eosin (HE) staining.

The myocardial tissues were collected and fixed in 10% formalin for 24 h. Next, the tissues were dehydrated, embedded, and cut into 5-μm-thick sections by a slicing machine (Leica Microsystems, Germany). The slides were baked in an oven at 60 °C for 3 h and stained with HE. We used an optical microscope (BX53, Olympus, Japan) to observe the sections.

TUNEL staining.

Three weeks after reperfusion, the rats were sacrificed. The myocardial tissue sections were used for terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) using an In Situ Apoptosis Detection Kit (40308ES20) according to the manufacturer's instructions. The samples were baked in an oven at 60 °C for 3 h, washed with xylene three times (20 min each time), dehydrated with absolute ethanol for 5 min twice, followed by serial ethanol rinses (95% ethanol, 90% ethanol, and 80%

ethanol each for 5 min), and the slides incubated in Proteinase K for 20 min before washing with PBS three times (5 min each time). The sections were stained with 4', 6-diamidino-2-phenylindole (DAPI) and washed with PBS four times (5 min each time). Finally, we used a light microscope to observe the collected images.

Western blotting.

Total proteins were obtained from the myocardial tissue, which was first sheared and placed in a 2 ml EP tube and immersed in the RIPA lysis buffer with phenylmethyl sulfonylfuoride (PMSF, Beyotime, Shanghai, China). The EP tube was placed in a tissue homogenizer for 10 min and lysed for 30 min on ice. After homogenization with an ultrasonic homogenizer at 4 °C, centrifugation was performed at 12000 rpm for 5 min. The supernatant was the total protein. Next, we used the bicinchoninic acid assay kit (Beyotime, Shanghai, China) to determine the concentration of protein. After denaturation of total protein, all membranes were blocked with 5% skim milk for 120 min, and then incubated at 4 °C overnight with primary antibodies: anti-CHOP (1:500), anti-PERK (1:1000), anti-Bcl-2 (1:1000), anti-Bax (1:2000), anti-caspase3 (1:1000), anti-ATF4 (1:500), and anti-β-actin (1:500). After washing with TBST 5 times (5 min each time), all membranes were incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody (1:50000) for 2 h at room temperature. The enhanced chemiluminescence (ECL) system (Applygen, Beijing, China) was applied to detect immunoreactive bands. After scanning, quantitative analysis was carried out with BandScan. The β-actin antibody was used as an internal reference.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR).

Total RNA was extracted from myocardial tissues using the TRIzol method (BOYAO, Shanghai, China) according to the manufacturer's instructions. The concentration and purity of total RNA were determined by measuring the OD_{260} and the OD_{260}/OD_{280} ratio. The gene expression levels of CHOP, ATF4, PERK, and β-actin were measured by RT-PCR as described previously [27]. The expression of each mRNA was calculated using the $2 - \Delta\Delta Ct$ method. β-actin was used as an internal reference. The primers used in this paper are listed in Table 1.

Table 1
Primers of ATF4, CHOP, PERK and β-actin

Gene	Primer	Sequence (5’–3’)	PCR Products (bp)
ATF4	Forward	ATTCTTGCAGCCTCTTCCCT	213
	Reverse	AGGTAGGACTCAGGGCTCAT	
CHOP	Forward	TACTCTTGACCCTGCATCCC	170
	Reverse	ACTGACCACTCTGTTTCCGT	
PERK	Forward	AGTCGGTCTTTCTCAGTGGG	160
	Reverse	CCATGTCGCAATCTGTCAGG	
β-actin	Forward	CACGATGGAGGGGCCGGACTCATC	240
	Reverse	TAAAGACCTCTATGCCAACACAGT	

Statistical analysis.

SPSS 17.0 statistical software was used for the analysis of all experimental data. Groups were compared using one-way analysis of variance (ANOVA). All experimental data were expressed as mean ± SEM. $P < 0.05$ was considered significant.

Results

Effects of RDN and ARNI on I/R injury-induced cardiac dysfunction

One week after I/R, we used echocardiography to assess the changes in cardiac function in each group. We found no significant changes in the HR, systolic pressure, or diastolic pressure (Fig. 2(A)). I/R injury significantly decreased LVEF and LVFS and increased LVSD and LVDd compared to the normal and sham groups. Figure 2 (B) shows the changes in each group 3 weeks after I/R. The I/R+RDN and I/R+ARNI groups have significantly ameliorated LVEF and LVFS and reversed expansion of the LVSD and LVDd compared to the I/R group. We inferred that RDN and ARNI can improve the cardiac function in rats with I/R injury. We also found that ARNI was slightly superior to RDN. In addition, the HR in the I/R group was slightly decreased compared to the other groups. We found no significant changes in the systolic pressure and diastolic pressure response.

Effects of RDN and ARNI on hormone activity

Serum NE, Ang II, and ALD levels were significantly increased in the I/R group (Fig. 3(A)), but decreased in the I/R+RDN and I/R+ARNI groups. RDN had a better effect on NE level than ARNI treatment, but the ARNI treatment showed a better effect on the levels of Ang II and ALD than RDN.

HE staining

HE staining was performed to assess the microstructural changes in cardiomyocytes in a cross-section of the heart. In the I/R group, edema of the cardiomyocytes was obvious, the myofilament arrangement was disordered, and degradation and necrosis were accompanied by inflammatory cell infiltration and hemorrhage. However, in the I/R+RDN and I/R+ARNI groups, we the myocardial tissue edema was alleviated and the abnormalities in the myofilaments were ameliorated (Fig. 3(B)), indicating that RDN and ARNI can alleviate I/R injury.

TTC staining

The infarct size was smaller in the I/R+RDN and I/R+ARNI groups compared to the I/R group (Fig. 4). No infarct was found in the normal group, sham group, or RDN group.

TUNEL staining

We used TUNEL staining to detect cardiomyocyte apoptosis in all groups. Three weeks after I/R, apoptosis of cardiomyocytes and fibroblasts in myocardial tissue increased significantly in the I/R group (Fig. 5(A)), which also had a significantly higher level of apoptosis than the other groups. This was greatly diminished by RDN and ARNI (Fig. 5(B)). ARNI was also more effective than RDN at reducing cardiomyocyte apoptosis. Therefore, RDN and ARNI can reduce cell apoptosis in I/R injury.

Expression of apoptosis-related proteins

The protein expression levels of Bax and caspase-3 were significantly higher in the I/R group than in other groups (Fig. 6), whereas the protein expression level of Bcl-2 was significantly lower in the I/R group. Compared to the I/R group, the protein expression levels of Bax and caspase-3 were significantly lower in the I/R+RDN and I/R+ARNI groups, whereas the protein expression level of Bcl-2 protein was significantly increased. This indicates that treatment with RDN and ARNI can improve cardiomyocyte apoptosis. Furthermore, we found that ARNI was superior to RDN.

Effect of RDN on the PERK/ATF4 signaling pathway

To investigate whether RDN affected cardiomyocyte apoptosis through regulation of the ERS PERK/ATF4 signaling pathway in I/R injury, Western blotting and RT-PCR were used to determine the protein and mRNA expression levels of PERK, ATF4, and CHOP in the five groups (Fig. 7 and 8). PERK, ATF4, and CHOP expression levels increased significantly in the I/R group compared to the other groups, whereas the levels in the I/R+RDN group decreased compared to the I/R group. Therefore, we speculated that RDN may reduce apoptosis by inhibiting the ERS PERK/ATF4 signaling pathway.

Discussion

Myocardial I/R injury is a common pathophysiological process in the treatment of cardiovascular diseases, and is an important reason behind aggravated myocardial damage and arrhythmia. The mechanism of I/R has not yet been fully elucidated. It is generally thought that oxygen free radicals and intracellular calcium overload are the main mechanisms involved in I/R injury.

The endoplasmic reticulum (ER) is an important organelle in the regulation of protein folding and Ca^{2+} homeostasis. When the body presents with hypoxia, sugar deficiency, ischemia, large amount of free radical accumulation, Ca^{2+} homeostasis, or other stress conditions, it can cause ER disorder, leading to apoptosis (i.e., ERS)). Many studies have confirmed that I/R injury is closely related to ERS. One study found that apoptosis is the main manifestation of I/R injury, and with the occurrence of reperfusion injury, ERS can activate the related apoptosis signaling pathway, aggravate the cardiomyocyte apoptosis, and promote infarct expansion [28]. The PERK/ATF4 pathway, as one of the main pathways of ERS, induces apoptosis by activating the expression of downstream ATF-4 proapoptotic proteins [29].

Apoptosis is closely related to the caspase family, which is composed of a series of specific proteases acting on cysteine and aspartic acid, which are present in the cytoplasm in the form of proenzyme and activated by protease hydrolysis in response to various apoptosis signals. Activated caspase can act on a variety of target proteins, such as nuclear protein, signal transduction-related protein, and cytoskeletal protein. Thus, the cleavage of a variety of target proteins can eventually cause cell death. Bcl-2 and Bax are a pair of positive and negative regulators of apoptosis that are closely related to apoptosis caused by myocardial infarction. An increase in Bcl-2 protein expression inhibits apoptosis and leads to cell survival, whereas overexpression of Bax protein can cause cell death.

I/R injury is characterized by activation of the SNS and RAAS, which shows that the concentration of NE, Ang II, and ADL increases, leading to ventricular remodeling and heart failure [30]. NE has a direct toxic effect on cardiomyocytes. Ang II is a local growth factor and can stimulate cardiomyocyte hypertrophy, up-regulate cardiomyocyte protein synthesis and RNA expression, mediate up-regulation of inflammatory interleukin 6 (IL6), and promote myocardial fibrosis [31]. Aldosterone can cause necrosis of cardiomyocytes by causing secondary hypokalemia, then repair fibrosis [32] and promote myocardial remodeling, resulting in a series of pathophysiological changes in the myocardium [33].

The major mechanism of RDN is the removal of the afferent and efferent nerves of the kidney, which significantly reduces the overactive systemic sympathetic activity through the central nervous system feedback mechanism [34]. Sacubitril-valsartan is the first ARNI that can act as natriuretic diuretic or vasodilator, and prevent and reverse myocardial remodeling by simultaneously inhibiting angiotensin receptor and enkephalase. Some studies [35] speculate that ARNIs may have a beneficial effect on cardiac remodeling by inhibiting myocardial hypertrophy and fibrosis, offering better cardiac protection. However, whether ARNIs can delay cardiomyocyte apoptosis requires further study.

Our study confirmed that the NE, Ang II, and ALD levels increased significantly in the I/R group, but decreased significantly after RDN and ARNI treatment, indicating that both RDN and ARNI could inhibit the activity of the RAAS and SNS. In addition, RDN and ARNI can improve cardiac function and ventricular remodeling. As we found that the expression of Bax, caspase-3, CHOP, PERK, and ATF4 significantly increased in the I/R group, but the expression of Bcl-2 decreased, with recovery to varying degrees after RDN, we concluded that I/R can promote caspase-3 activation and induce apoptosis, whereas RDN can inhibit apoptosis by regulating the PERK/ATF4-mediated apoptosis pathway.

Our study has several limitations. First, our surgical technique for RDN is different from clinical catheter ablation and does not fully simulate clinical approaches. Second, because of the small sample size, we need to expand the experimental sample size to confirm our conclusion and further explore the effect of RDN on the ERS PERK/ATF4 signaling pathway. Third, our experimental period was 2 weeks, and we think that the effect of ARNI and RDN may be more significant with prolonged experimental duration.

Conclusion

In our study, RDN and ARNI ameliorated cardiomyocyte apoptosis in myocardial I/R injury, and ARNI appeared to be more effective than RDN in improving cardiomyocyte apoptosis. Thus, ARNIs may be a therapeutic strategy for I/R patients in the future. Furthermore, the effect of RDN may be associated with regulation of the ERS PERK/ATF4 signaling pathway.

Limitations

Several limitations were present in our study. (1)our surgical technique of performing RDN is different from clinical catheter ablation and does not fully simulate clinical approaches. (2)Because of the small sample size, we need to expand the experimental sample size to confirm our conclusion and further explore the effect of RDN on endoplasmic reticulum stress PERK/ATF4 Signaling Pathway. (3) Our experimental study time is 2 weeks, We think that the effect of ARNIs and RDN may be more significant with the prolongation of experimental time.

Abbreviations

ACEI: Angiotensin-converting enzyme inhibitor; ALD: Aldosterone; AMI: Acute myocardial infarction; ANOVA: Analysis of variance; ARB: Angiotensin II receptor blocker; ARNIs: Angiotensin receptor neprilysin inhibitors; ATF4: Activating transcription factor 4; ATF6: Activating transcription factor 6; CHOP: C/EBP-homologous protein; DAPI: 4', 6-diamidino-2-phenylindole; ECG: Electrocardiogram; ELISA: Enzyme-linked immunosorbent assay; ERS: Endoplasmic reticulum stress; HE: Hematoxylin-eosin; HR: Heart rate; IL6: Interleukin 6; I/R: Ischemia-reperfusion injury; IRE-1: Inositol-requiring protein-1; LAD: Left anterior descending coronary artery; LVDd: Left ventricular end-diastolic diameter; LVEF: Left ventricular ejection fraction; LVFS: Left ventricular fractional shortening; LVSD: Left ventricular end-systolic diameter; NE: Norepinephrine; PERK: Protein kinase RNA-like ER kinase; PMSF: Phenylmethyl sulfonylfuoride; PRC: People's Republic of China; RAAS:Renin-angiotensin-aldosterone system; RDN: renal denervation; RT-PCR: Reverse transcription-polymerase chain reaction; SNS: Sympathetic nervous system; SPF: Specific pathogen free; TTC: 2,3,5-Triphenyltetrazolium chloride; TUNEL: Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling.

Declarations

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated during the current study.

Author Contributions

Zheng Zhao was responsible for the study design, data analysis, statistical analysis manuscript preparation and editing; Faquan Li was responsible for the literature research; Chengzhi Lu was responsible for planning, technical, funding, and mentoring support of this project. Yiyao Jiang was responsible for technical support of this project.

Competing interests

The authors have no conflicts of interest to declare.

Consent for publication

Not applicable.

Ethics approval

All experimental protocols were approved by the local animal care and use committee (the Animal Experimental Ethics Association of Tianjin First Centre Hospital). The methods were carried out in accordance with the approved guidelines.

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Figures

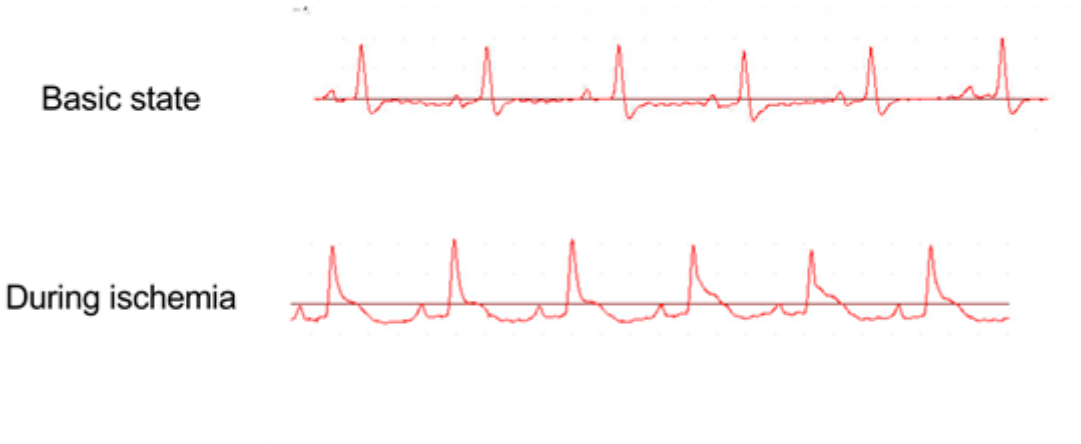

Figure 1

Electrocardiography at different time points.

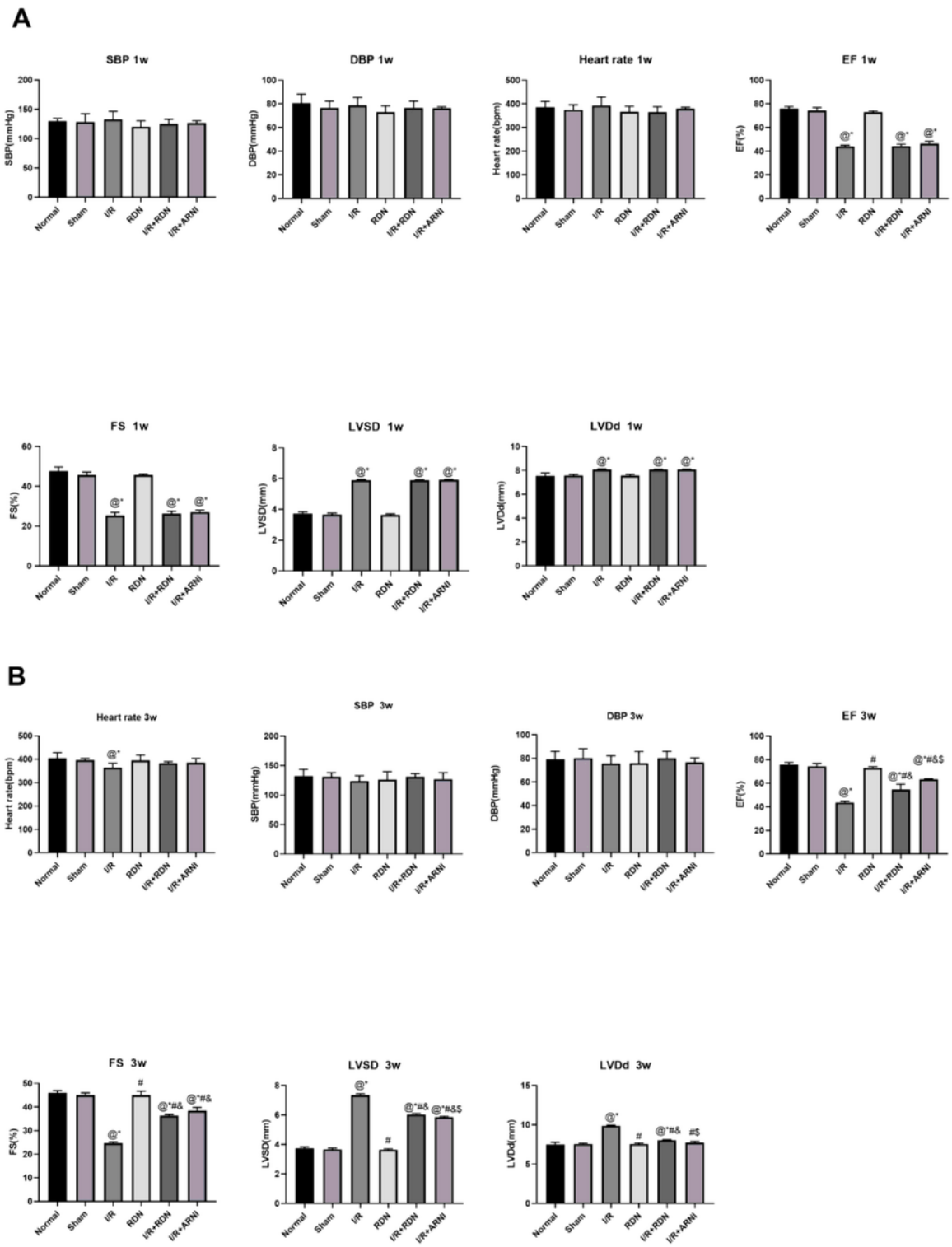

Figure 2

Echocardiographic assessment of cardiac function in rats from each group 1 week and 3 weeks after I/R. RDN and ARNI improved heart function. (A) The heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), left ventricular systolic diameter (LVSD), and left ventricular end-diastolic diameter (LVDd) (N = 10 in each group) after I/R for 1 week recorded by echocardiography. (B) The levels of SBP, DBP, HR, EF, FS, LVSD,

and LVDd after I/R for 3 week recorded by echocardiography. Data are given as mean ± SEM. @P < 0.05 vs. Normal group, *P < 0 05 vs. sham group by one-way ANOVA.

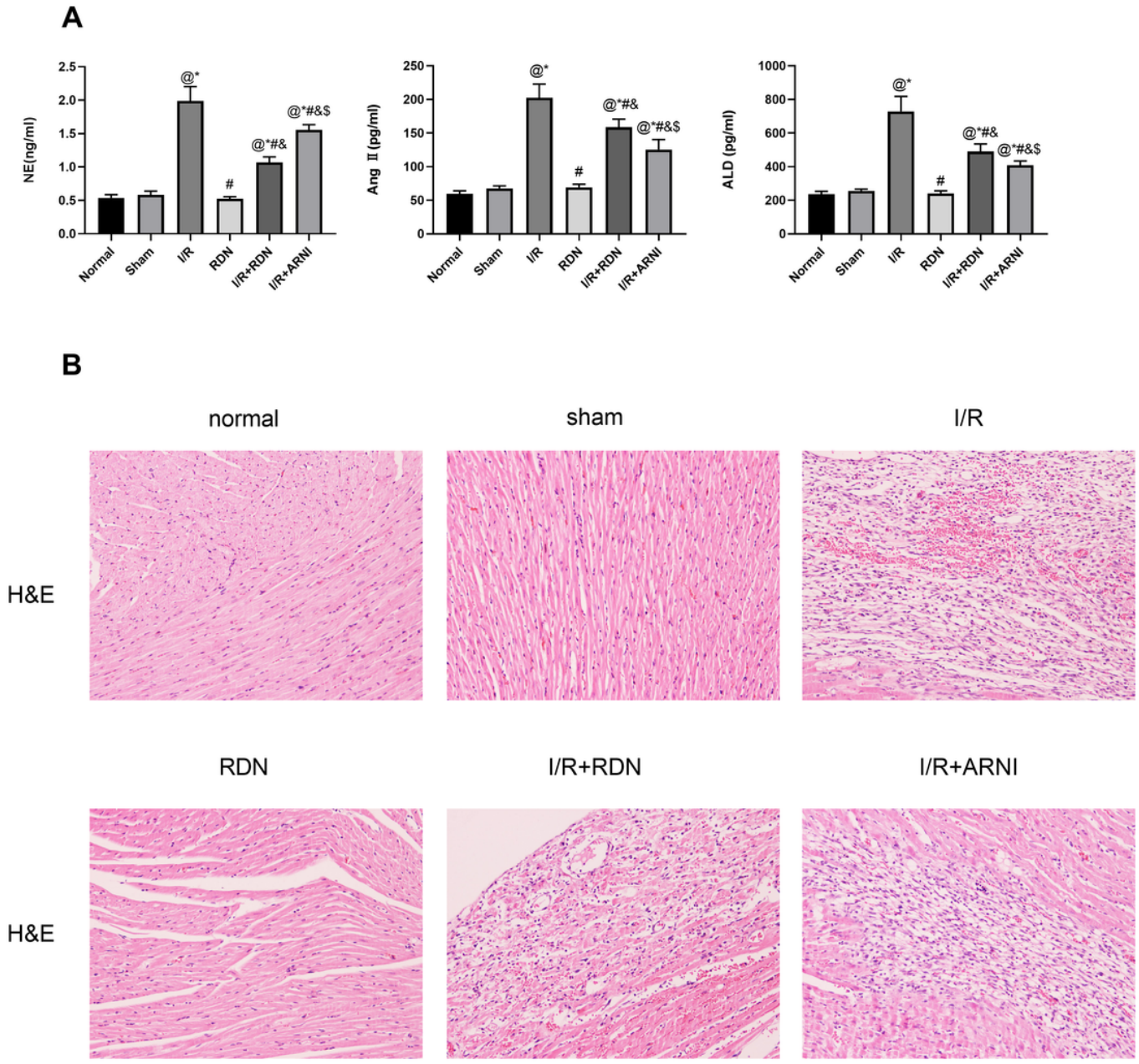

Figure 3

RDN and ARNI reduced inflammatory cell infiltration in the heart. (A) Histopathological changes in the myocardium observed by HE staining (200×). (B) NE, Ang II, and ALD were determined by ELISA in each group. @P<0.05 vs. normal group, *P<0.05 vs. sham group, #P<0.05 vs. I/R group, &P<0.05 vs. RDN group, $P<0.05 vs. I/R+RDN group.

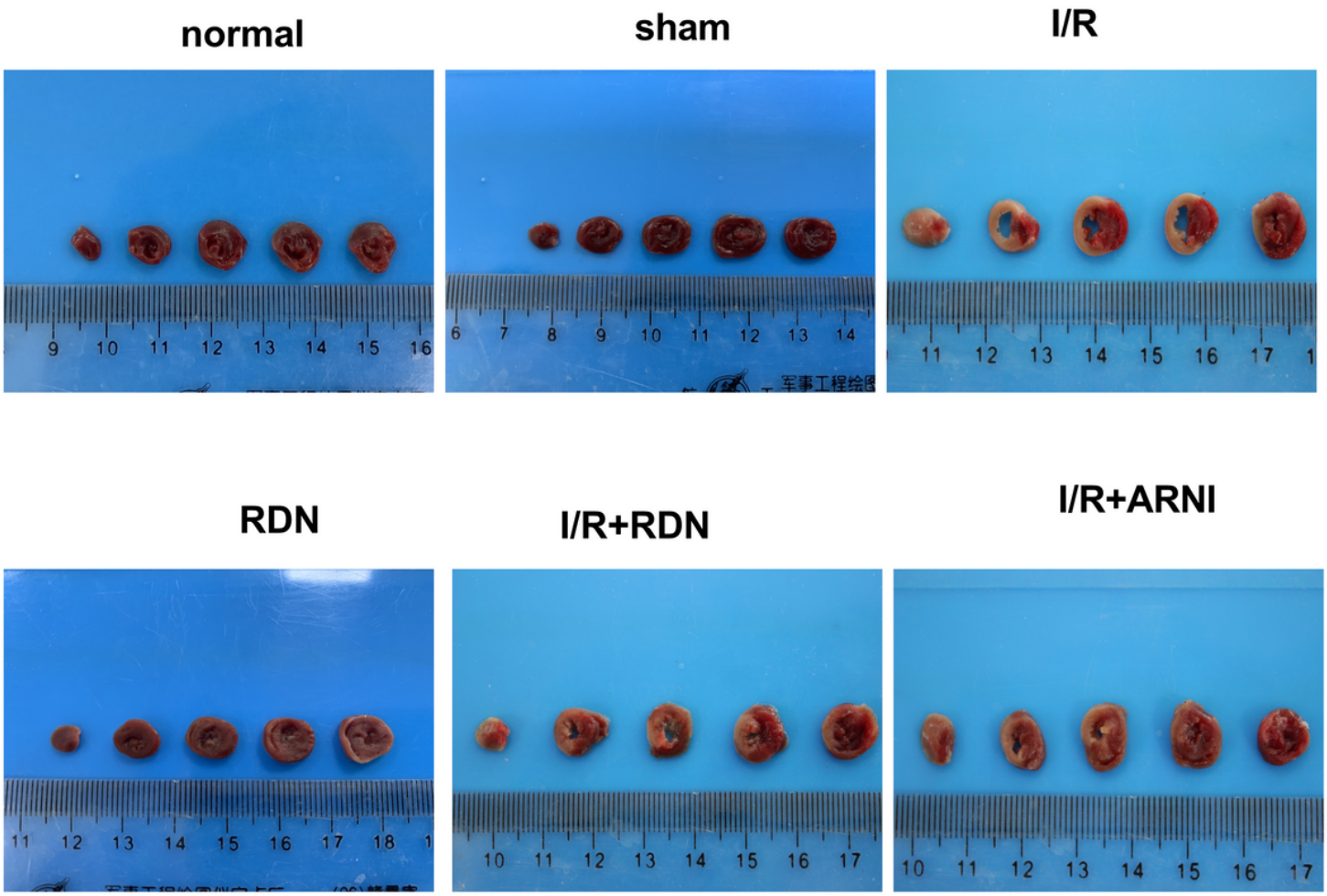

Figure 4

Representative pictures of TTC staining in all groups. The infarct area is stained white, and the non-infarct area is stained red.

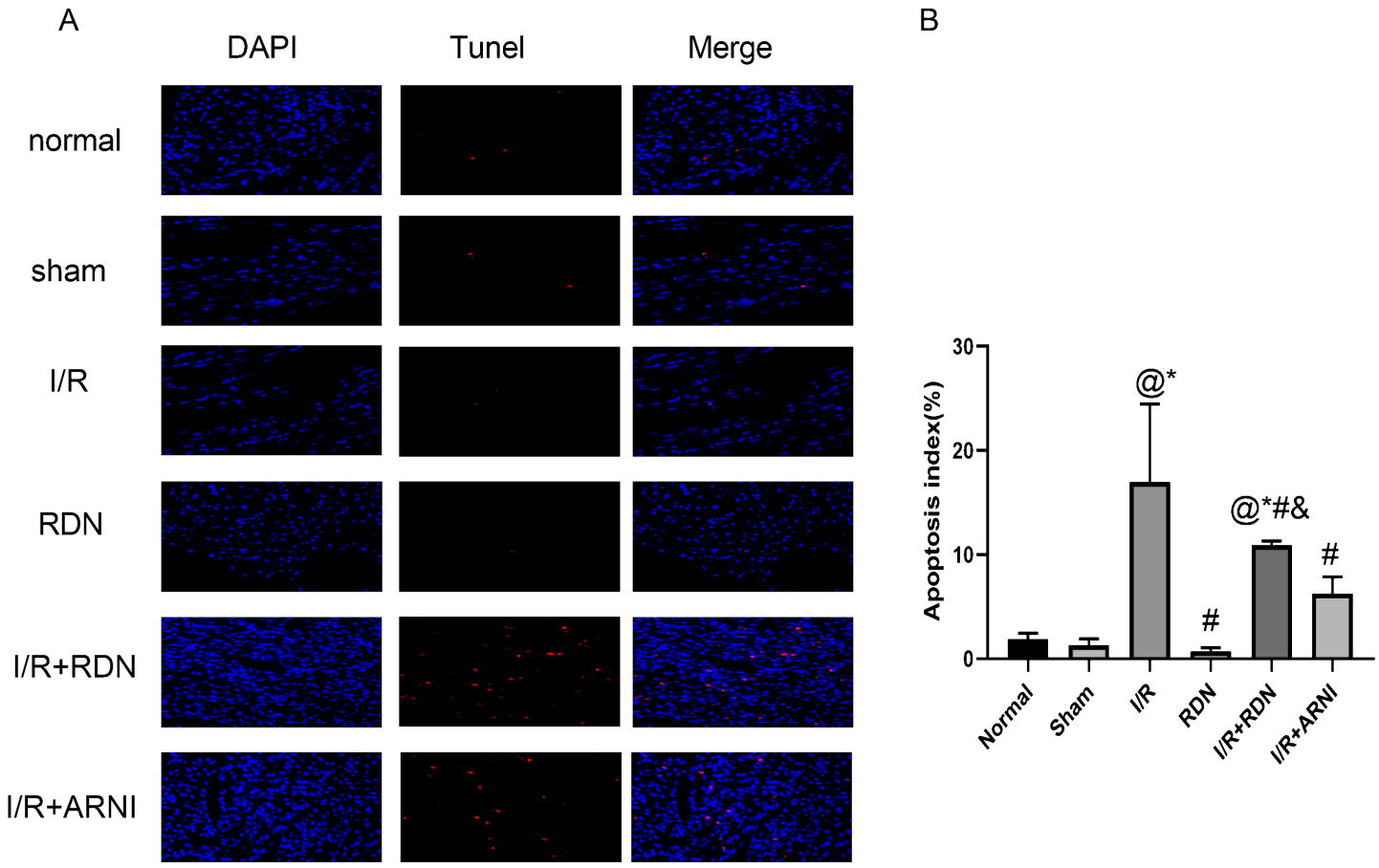

Figure 5

RDN and ARNI can reduce cell apoptosis in myocardial ischemia. (A) Representative sections from heart tissues after reperfusion for the detection of nuclear DNA fragmentation performed by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) staining. Images were obtained at 400×. (B) Quantitative analysis of the proportion of TUNEL-positive cells in heart tissues from each group. 1.91% in normal group, 1.32% in sham group, 16.98% in I/R group, 0.68% in RDN group, 10.90% in I/R+RDN group, 6.25% in I/R+ARNI group. @$p<0.05$ vs. normal group, *$p<0.05$ vs. the sham group, #$p<0.05$ vs. I/R group, &$p<0.05$ vs. RDN group.

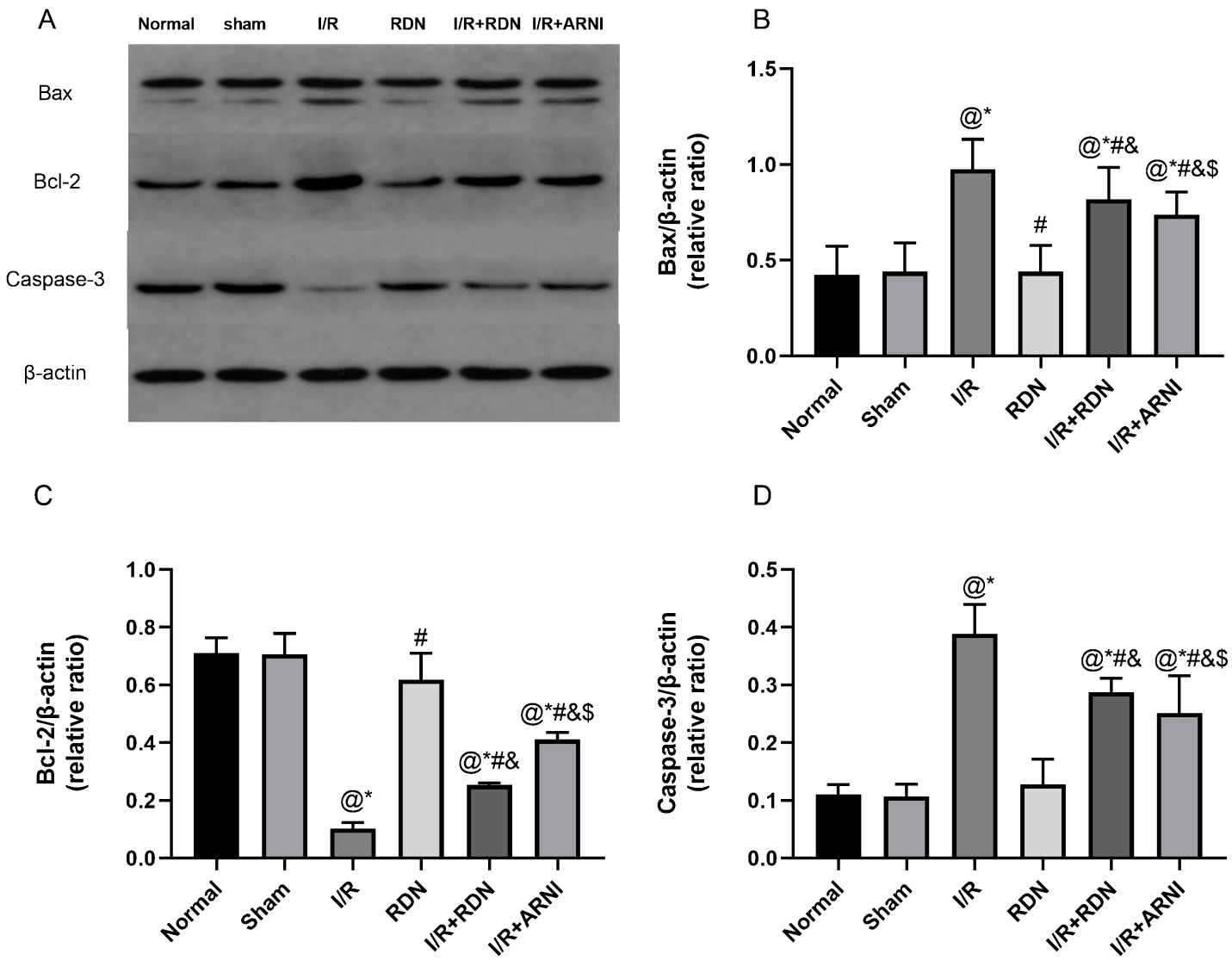

Figure 6

Apoptosis-related protein expression. (A) Protein analysis of in vitro samples. (B-D) The expression of Bax, Bcl-2, and caspase-3 in six groups determined by Western blotting. @p<0.05 vs. normal group, *p<0.05 vs. sham group, #p<0.05 vs. I/R group, &p<0.05 vs. RDN group, $p<0.05 vs. I/R+RDN group.

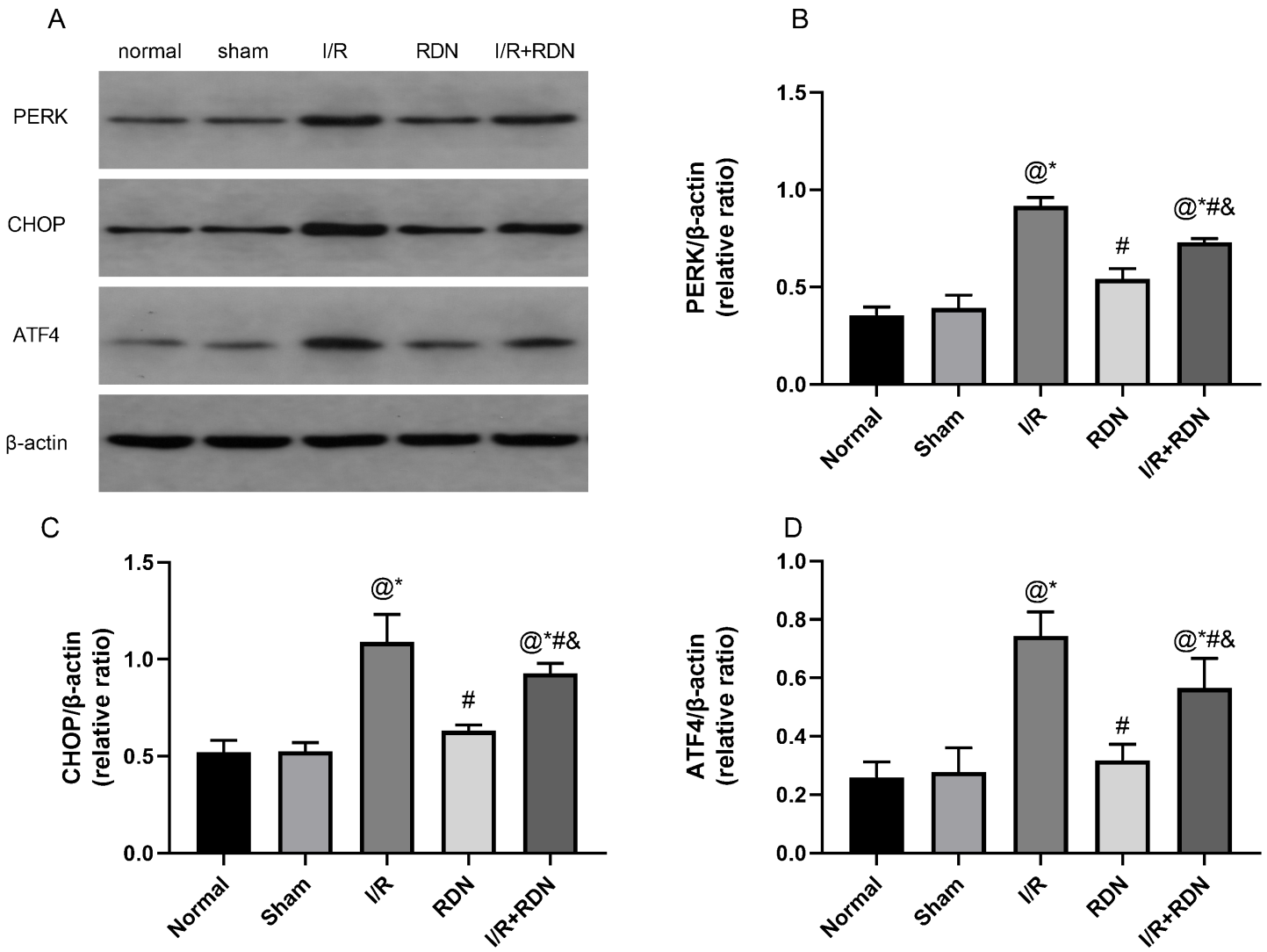

Figure 7

PERK/ATF4 signaling-related protein expression. (A) Protein analysis of in vitro samples. (B-D) The expression of PERK, CHOP, and ATF4 in five groups determined by Western blotting. @p<0.05 vs. normal group, *p<0.05 vs. sham group, #p<0.05 vs. I/R group, &p<0.05 vs. RDN group.

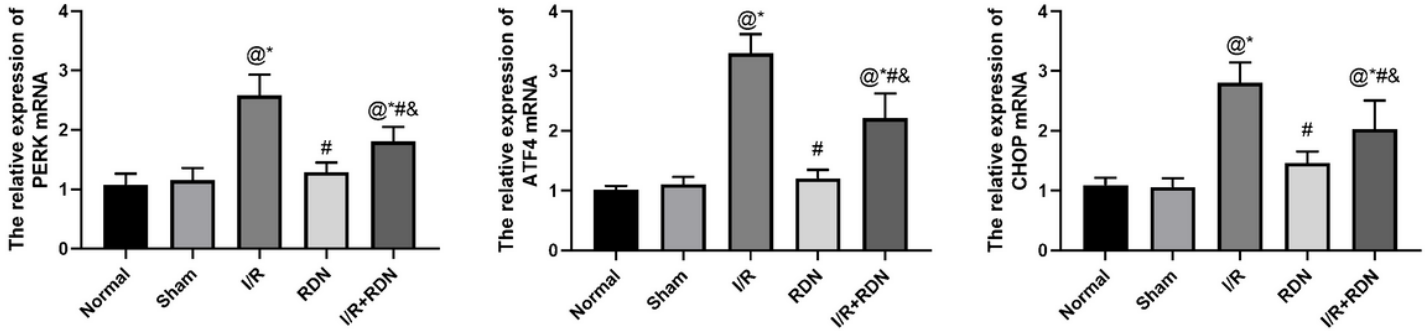

Figure 8

Expression of PERK, ATF-4, and CHOP in five groups determined by RT-PCR. @p<0.05 vs. normal group, *p<0.05 vs. sham group, #p<0.05 vs. I/R group, &p<0.05 vs. RDN group.