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Renal Denervation Ameliorates Cardiomyocyte Apoptosis in Myocardial Ischemia–Reperfusion Injury Through Regulating Mitochondria–Endoplasmic Reticulum Contact

ABSTRACT

Background: Myocardial ischemia–reperfusion injury (I/R) has been improved with drugs and effective reperfusion, but it still cannot be prevented.

Methods: To investigate whether renal denervation (RDN) reduces cardiomyocyte apoptosis by ameliorating endoplasmic reticulum stress, 60 male specific pathogen-free (SPF) Wistar rats were randomly divided into 6 groups (n = 6). We established the I/R rat model by ligating the left anterior descending artery. The I/R+ angiotensin receptor neprilysin inhibitors (ARNI) group received ARNIs for 2 weeks until euthanasia.

Results: The I/R+RDN and I/R+ARNI groups have signifi antly ameliorated left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) and reversed expansion of the left ventricular end-systolic diameter (LVSD) and left ventricular end diastolic diameter (LVDD) compared to the I/R group. The levels of norepinephrine (NE), angiotensin II, and aldosterone (ALD) increased signifi antly in the I/R group, but decreased signifi antly after RDN and ARNI intervention. In the I/R+RDN and I/R+ARNI groups, the myocardial tissue edema was alleviated. The infarct size was smaller in the I/R+RDN and I/R+ARNI groups compared to the I/R group. Apoptosis of cardiomyocytes and fib oblasts in myocardial tissue increased signifi antly in the I/R group, which was greatly diminished by RDN and ARNI. The expression of Bax, caspase-3, CHOP, PERK, and ATF4 protein was signifi antly 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: The effect of RDN may be associated with regulating the endoplasmic reticulum stress PERK/ATF4 signaling pathway.

Keywords: Myocardial ischemia–reperfusion injury, renal denervation, ARNI, endoplasmic reticulum stress, cardiomyocyte apoptosis

ORIGINAL INVESTIGATION

INTRODUCTION

Acute myocardial infarction (AMI) remains a leading cause of mortality and morbidity worldwide.[1] Acute myocardial infarction 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 fl w.[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.

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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] Renal denervation can block renal transmission nerves, specifi ally and effectively reducing the activity of the SNS and RAAS, improving cardiac function and ventricular remodeling.[10] Currently, we know that norepinephrine and angiotensin II (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 at enuates cardiac fib osis.

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 fib osis.[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.

METHODS

Experimental Animals and Treatment

Sixty healthy adult male specific pathogen free (SPF) Wistar 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 Animals and were approved by the Laboratory Animal Ethics Committee (Institute of Radiation Medicine, Chinese Academy of Medical Sciences). 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 : darkness, 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 6 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 euthanasia. The doses used in the experiment were based on a previous study.[24] We found no signifi ant differences in breed, body weight, age, or sex among the 6 groups. All rats were sacrifi ed using an i.p. injection of an overdose of pentobarbital sodium.

Ischemia–Reperfusion Injury Procedures

The I/R model was established similar to a previous study.[25] Briefl , the rats were anesthetized using an R540 series anesthetic machine (RWD Life Science, Shenzhen, China) and fi ed 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 minutes. The myocardium turned white, and their condition was confirmed by ST segment elevation in lead II of the electrocardiogram (ECG; Figure 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.

Renal Denervation 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,

HIGHLIGHTS

- RDN (Renal Denervation) and ARNI (Angiotensin Receptor Neprilysin Inhibitors) ameliorated cardiomyocyte apoptosis in myocardial I/R injury.
- ARNI appeared to be more effective than RDN in improving cardiomyocyte apoptosis.
- ARNIs may be a therapeutic strategy for I/R patients in the future. The effect of RDN may be associated with regulation of the ERS PERK/ATF4 signaling pathway.

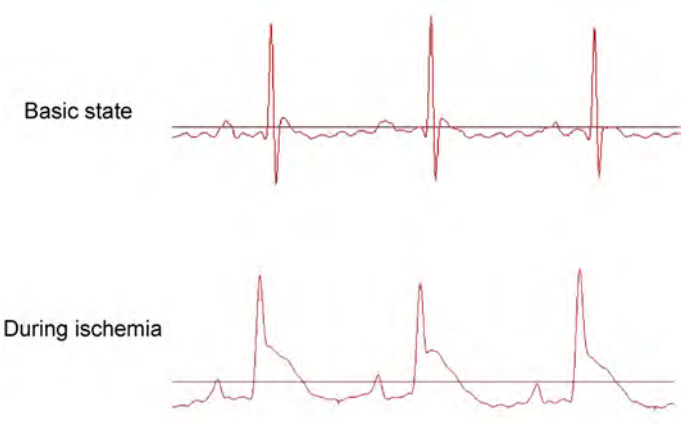

Figure 1. Electrocardiography at different time points. Basic state: normal electrocardiogram. During ischemia: the ST segment was elevated after ligation.

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 vessel with phenol (10% phenol in 95% ethanol) using a cotton swab for 2 minutes to destroy the remaining nerves. The rats in the sham group were painted with 0.9% saline without destruction of the bilateral renal nerves.

Angiotensin Receptor Neprilysin Inhibitor Procedures

One week after reperfusion, the rats in 1 subgroup were fed ARNIs (60 mg/kg) sacubitril/valsartan (LCZ696) (SML1380; Sigma-Aldrich; Merck KGaA) for 2 weeks and then sacrifi ed. 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 4-channel physical recorder (BL-420F systems, Chengdu Technology and Market, China) was used to monitor the ECG signals.

Enzyme Immunoassay of Norepinephrine, Angiotensin II, and Aldosterone

After 3 weeks of reperfusion, blood samples were collected and centrifuged for 30 minutes at 3000 *g*. Ischemic myocardial tissue was also collected and centrifuged for 10 minutes 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 Staining

At the end of the reperfusion, the rats were sacrifi ed immediately for 2,3,5-triphenyltetrazolium chloride (TTC) staining. The hearts were removed and washed with physiological saline solution. The myocardial tissues were frozen at −20°C for 20 minutes and sliced into 2 mm-thick sections. After incubation with 1% TTC solution (Beyotime, Shanghai, China) at 37°C for 15 minutes, each section was photographed. The infarct area was stained white, and the non-infarct area was stained red.

Hematoxylin–Eosin Staining

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

Terminal Deoxynucleotidyl Transferase 2′-Deoxyuridine, 5′-Triphosphate Nick End Labeling Staining

Three weeks after reperfusion, the rats were sacrifi ed. 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 hours, washed with xylene 3 times (20 minutes each time), dehydrated with absolute ethanol for 5 minutes twice, followed by serial ethanol rinses (95% ethanol, 90% ethanol, and 80% ethanol each for 5 minutes), and the slides were incubated in proteinase K for 20 minutes before washing with phosphate buffer saline (PBS) 3 times (5 minutes each time). The sections were stained with 4′,6-diamidino-2-phenylindole (DAPI) and washed with PBS 4 times (5 minutes 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 fir t sheared and placed in a 2 mL EP tube and immersed in the RIPA lysis buffer with phenylmethyl sulfonyl fluoride (PMSF, Beyotime, Shanghai, China). The EP tube was placed in a tissue homogenizer for 10 minutes and lysed for 30 minutes on ice. After homogenization with an ultrasonic homogenizer at 4°C, centrifugation was performed at 12000 rpm for 5 minutes. 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 minutes, 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 minutes each time), all membranes were incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody (1:50000) for 2 hours 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.

Ribonucleic Acid Extraction and Reverse Transcription Polymerase Chain Reaction

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.

Statistical Analysis

SPSS 17.0 statistical software and GraphPad prism 10.0 were used for the analysis of all experimental data. Before comparing the difference among each group, we used K-S analysis to test the normality of the data in each group. Results showed that data are distributed normally in all groups, so the one-way analysis of variance (ANOVA) was used to compare the difference of each group. All experimental data are expressed as mean ± standard deviation (SD). $P < .05$ was considered signifi ant.

RESULTS

Effects of Renal Denervation and Angiotensin Receptor Neprilysin Inhibitor on Ischemia–Reperfusion 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 signifi ant changes in the HR, systolic pressure, or diastolic pressure (Figure 2A). I/R injury signifi antly decreased LVEF and LVFS and increased LVSD and LVDd compared to the normal and sham groups. Figure 2B shows the changes in each group 3 weeks after I/R. The I/R+RDN and I/R+ARNI groups have signifi antly 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 signifi antly different compared to the other groups. We found no signifi ant changes in the systolic pressure and diastolic pressure response.

Table 1. The primer of ATF4, CHOP, PERK, and β-actin

Gene name	Primer sequence (5′-3′)
ATF4	Forward: ATTCTTGCAGCCTCTTCCCT Reverse: AGGTAGGACTCAGGGCTCAT
CHOP	Forward: TACTCTTGACCCTGCATCCC Reverse: ACTGACCACTCTGTTTCCGT
PERK	Forward: ATGATGGTCTGCCAAGTGGG Reverse: CCATGTCGCAATCTGTCAGG
β-actin	Forward: ACGATGGAGGGGCCGGACTCATC Reverse: AAAGACCTCTATGCCAACACAGT

Effects of Renal Denervation and Angiotensin Receptor Neprilysin Inhibitor on Hormone Activity

Serum NE, Ang II, and ALD levels were signifi antly increased in the I/R group (Supplementary Figure 1), but decreased in the I/R+RDN and I/R+ARNI groups. RDN had a better effect on NE levels than ARNI treatment, but the ARNI treatment showed a better effect on the levels of Ang II and ALD than RDN.

Hematoxylin–Eosin Staining

Hematoxylin–eosin 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 myofilame t arrangement was disordered, and degradation and necrosis were accompanied by infla - matory cell infilt ation and hemorrhage. However, in the I/R+RDN and I/R+ARNI groups, the myocardial tissue edema was alleviated, and the abnormalities in the myofilame ts were ameliorated (Figure 3A), indicating that RDN and ARNI can alleviate I/R injury.

2,3,5-Triphenyltetrazolium Chloride Staining

The infarct size was smaller in the I/R+RDN and I/R+ARNI groups compared to the I/R group (Figure 3B). After quantitative analysis, no infarct was found in the normal group, sham group, or RDN group (Supplementary Figure 2).

Terminal Deoxynucleotidyl Transferase 2′-Deoxyuridine, 5′-Triphosphate Nick End Labeling Staining

We used TUNEL staining to detect cardiomyocyte apoptosis in all groups. Three weeks after I/R, apoptosis of cardiomyocytes and fib oblasts in myocardial tissue increased significantly in the I/R group, which also had a signifi antly higher level of apoptosis than the other groups. This was greatly diminished by RDN and ARNI (Figure 3C). 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 signifi antly higher in the I/R group than in other groups (Figure 4), whereas the protein expression level of Bcl-2 was signifi antly lower in the I/R group. Compared to the I/R group, the protein expression levels of Bax and caspase-3 were signifi antly lower in the I/R+RDN and I/R+ARNI groups, whereas the protein expression level of Bcl-2 protein was signifi antly increased. This indicates that treatment with RDN and ARNI can improve cardiomyocyte apoptosis. Furthermore, we found that ARNI was superior to RDN.

Effect of Renal Denervation 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 5 groups (Figure 5 and Supplementary Figure 3). PERK, ATF4, and CHOP expression

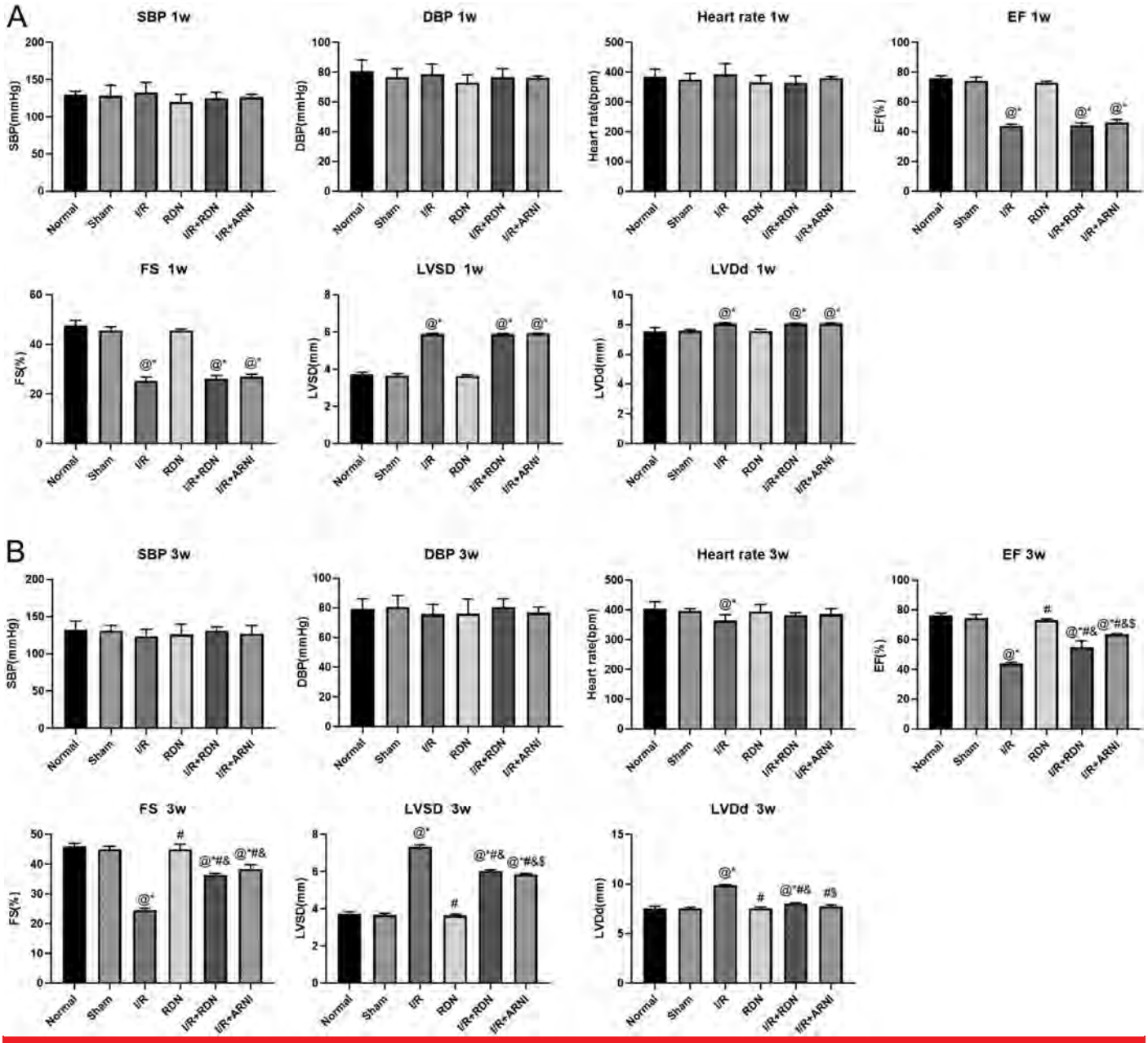

Figure 2. Echocardiographic assessment of cardiac function in rats from each group 1 week and 3 weeks after I/R. RDN and ARNI improved heart function. (A) The heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), left ventricular ejection fraction (LVEF), left ventricular fractional 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. Data are given as mean ± SEM. @*P* < .05 vs. Normal group, **P* < .05 vs. sham group by one-way analysis of variance.

levels increased signifi antly 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 deficienc , ischemia, a 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

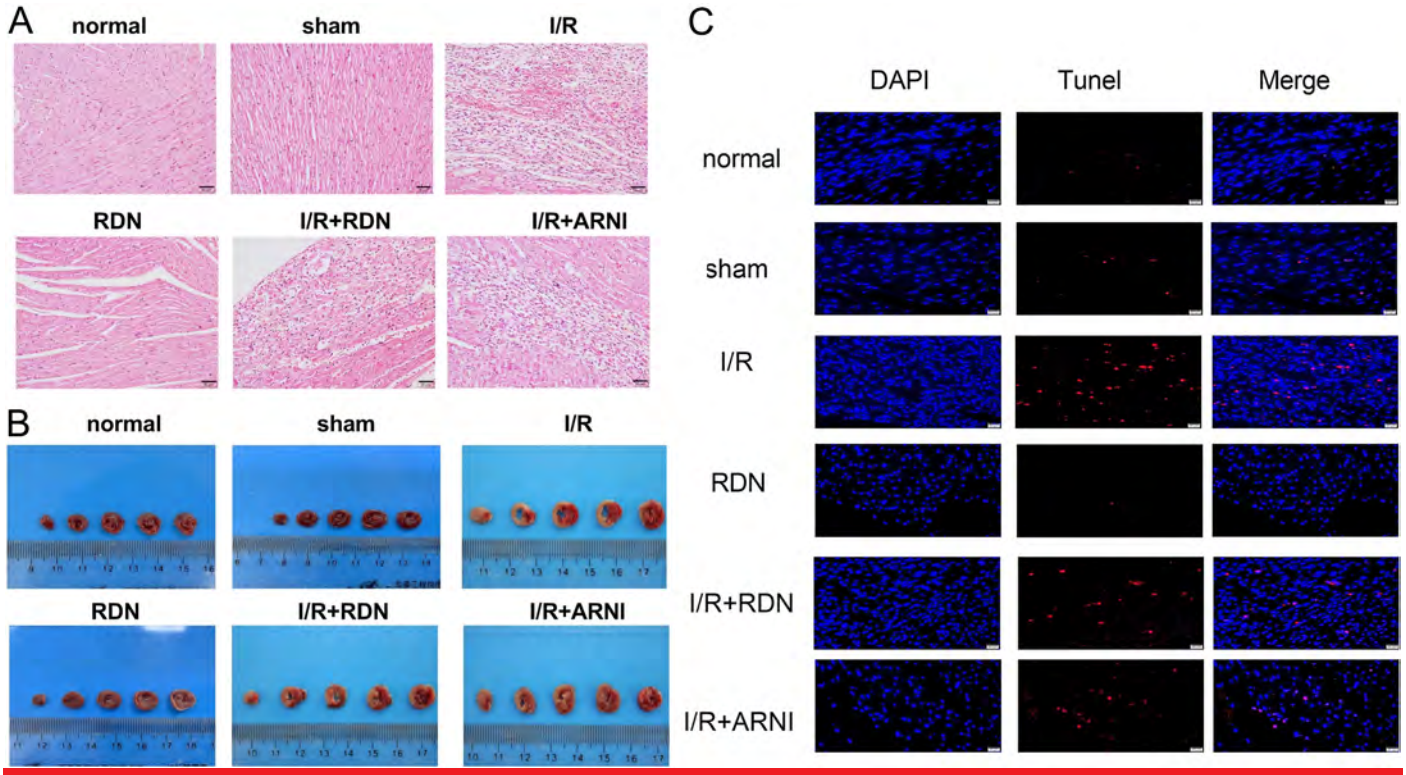

Figure 3. Representative images of HE staining, TTC staining, and TUNEL staining. (A) Histopathological changes in the myocardium observed by H&E staining (200×); (B) Representative pictures of TTC staining in all groups. The infarct area is stained white, and the non-infarct area is stained red. The infarct size in Each group had 0.20% in normal group, 0.40% in sham group, 57.88% in I/R group, 1.1% in RDN group, 49.70% in I/R+RDN group, 40.87% in I/R+ARNI group. $^{@}P < .05$ vs. normal group, $^{*}P < .05$ vs. the sham group, $^{\#}P < .05$ vs. I/R group, $^{\&}P < .05$ vs. RDN group, $^{\$}P < .05$ vs. I/R+RDN group; (C) Apoptosis was detected by TUNEL and the image was 400×.

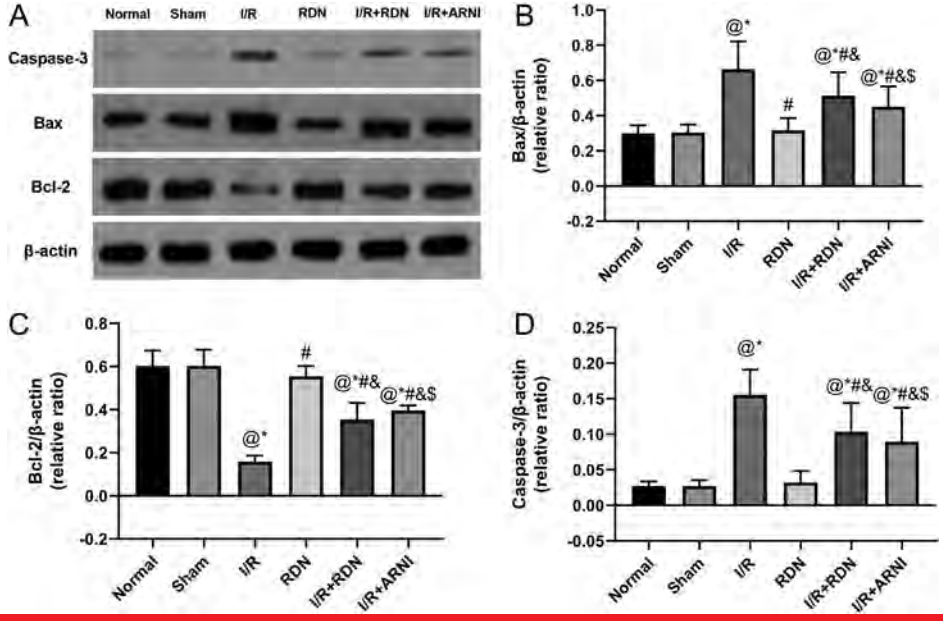

Figure 4. Apoptosis-related protein expression. (A) Protein analysis of in vitro samples. (B-–) The expression of Bax, Bcl-2, and caspase-3 in 6 groups determined by Western blotting. $^{@}P < .05$ vs. normal group, $^{*}P < .05$ vs. sham group, $^{\#}P < .05$ vs. I/R group, $^{\&}P < .05$ vs. RDN group, $^{\$}P < .05$ vs. I/R+RDN group.

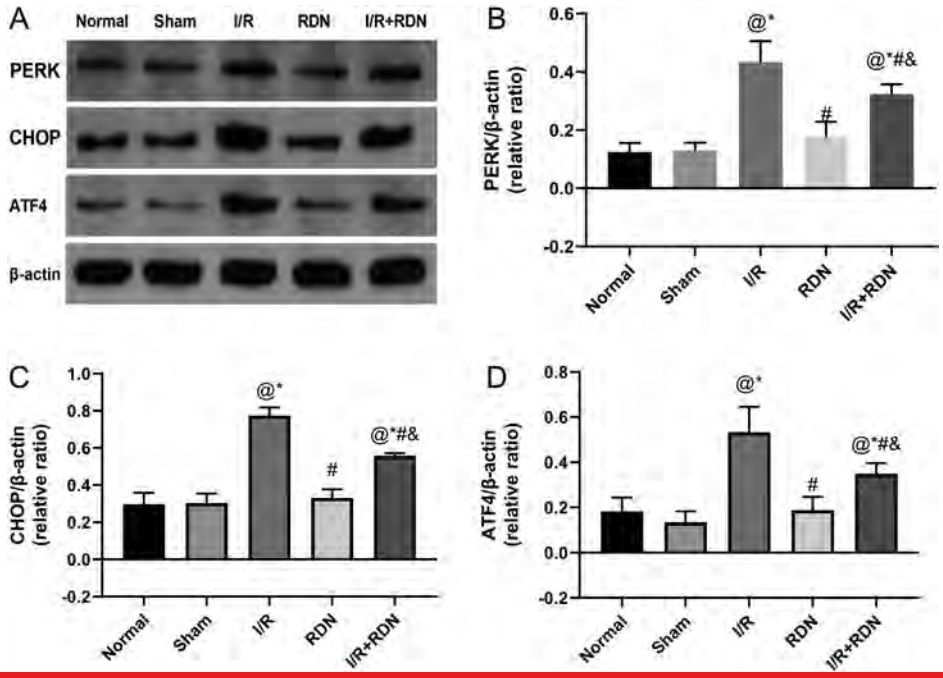

Figure 5. PERK/ATF4 signaling-related protein expression. (A) Protein analysis of *in vitro* samples. (B–D) The expression of PERK, CHOP, and ATF4 in 5 groups determined by Western blotting. $^{@}P < .05$ vs. normal group, $^{*}P < .05$ vs. sham group, $^{\#}P < .05$ vs. I/R group, $^{\&}P < .05$ vs. RDN group.

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.

Acute occlusion of the coronary artery leads to extensive myocardial necrosis, resulting in a sharp decrease in myocardial contractility, leading to cardiac functional deterioration and the occurrence of heart failure exacerbation. The increase in cardiac sympathetic nerve activity is the most damaging aspect of the sympathetic activation in heart failure.[30] Long-term activation of the sympathetic nervous system induces the expression of inflamma ory factors, promotes oxidative stress, myocardial cell hypertrophy, necrosis, apoptosis, and fib osis, leading to ventricular remodeling. Animal studies showed that inhibition of the sympathetic nervous system could confer protection against the damage to organs due to chronic excessive activation of sympathetic nerves.[31,32] 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.[33] NE has a direct toxic effect on cardiomyocytes. Angiotensin II is a local growth factor and can stimulate cardiomyocyte hypertrophy, up-regulate cardiomyocyte protein synthesis and RNA expression, mediate up-regulation of inflamma ory interleukin 6 (IL6), and promote myocardial fib osis.[34] Aldosterone can cause necrosis of cardiomyocytes by causing secondary hypokalemia, then repair fib osis[35] and promote myocardial remodeling, resulting in a series of pathophysiological changes in the myocardium.[36] Panico et al[37] considered that blocking SNS or RAS may be beneficial for improving the structure and function of the injured heart. Therefore, we established an I/R model to observe the improvement of cardiac function and ventricular remodeling after I/R by RDN. The major mechanism of RDN is the removal of the afferent and efferent nerves of the kidney, which signifi antly reduces the overactive systemic sympathetic activity through the central nervous system feedback mechanism.[38] Furthermore, RDN can reduce the inflamma ory response.[39] DiBona et al[40] demonstrated that RDN promotes the restoration of sodium excretion recovery in rats with decompensated heart failure. Wang et al[41] demonstrated that RDN can prevent and improve the post-MI deterioration of LV function and LV dilatation. These results demonstrate that RDN can inhibit cardiac structural remodeling, which is probably an effi acious treatment for heart failure.

Sacubitril–valsartan is the fir t ARNI that can act as a natriuretic diuretic or vasodilator, and prevent and reverse myocardial remodeling by simultaneously inhibiting the angiotensin receptor and enkephalase. Some studies[42] speculate that ARNIs may have a beneficial effect on cardiac remodeling by inhibiting myocardial hypertrophy and fib osis, offering better cardiac protection. Current research has confirmed that ARNI can inhibit neuroendocrine over-activation, exerting favorable sodium excretion, vasodilatation, diuresis, etc., thus promoting the improvement of the condition of heart failure.[43,44] ARNI has now become a category of recommendation for the treatment of patients with heart failure. The purpose of this trial is to clarify whether RDN is equally effective in improving cardiac function by comparing RDN with ARNI.

Our study confirmed that the NE, Ang II, and ALD levels increased signifi antly 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. In the present study, we found that RDN also had the same effect as ARNI in improving cardiac function.

As we found that the expression of Bax, caspase-3, CHOP, PERK, and ATF4 signifi antly 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 signifi ant with a prolonged experimental duration. Fourth, due to limited experimental conditions, the temperature of the rats was not monitored during the experiment, but a thermostatic blanket was used to keep the rats at a constant temperature during the operation.

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. This study confirmed that RDN has the effect of alleviating myocardial apoptosis. Based on this conclusion, we speculate that patients with hypertension complicated with ischemic cardiomyopathy who need RDN treatment may have more benefits than patients with hypertension alone. Furthermore, the effect of RDN may be associated with regulation of the ERS PERK/ATF4 signaling pathway.

Study 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 signifi ant with the prolongation of experimental time. (4) Due to limited experimental conditions, the temperature of the rats was not monitored during the experiment, but a thermostatic blanket was used to keep the rats at a constant temperature during the operation.

Availability of Data and Materials: Data sharing is not applicable to this article as no datasets were generated during the current study.

Ethics Committee Approval: All the experiments were conducted in accordance with the guide for the Care and the Use of Laboratory. The protocol was approved by Institute of Radiation Medicine, Chinese Academy of Medical Sciences (Approval number: IRM-DWLL-2021162).

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

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. Zheng Zhao and Faquan Li contributed equally to this work and should be considered co-fir t authors.

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Declaration of Interests: The authors have no conflicts of interest to declare.

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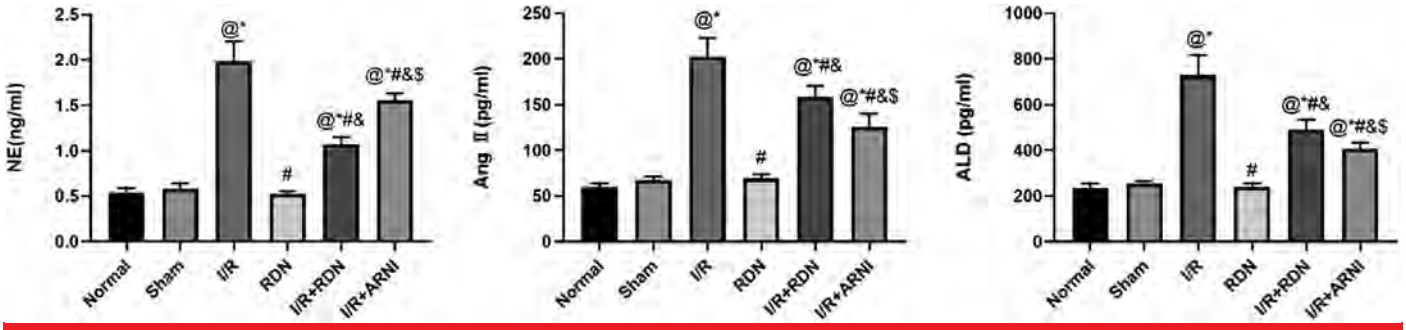
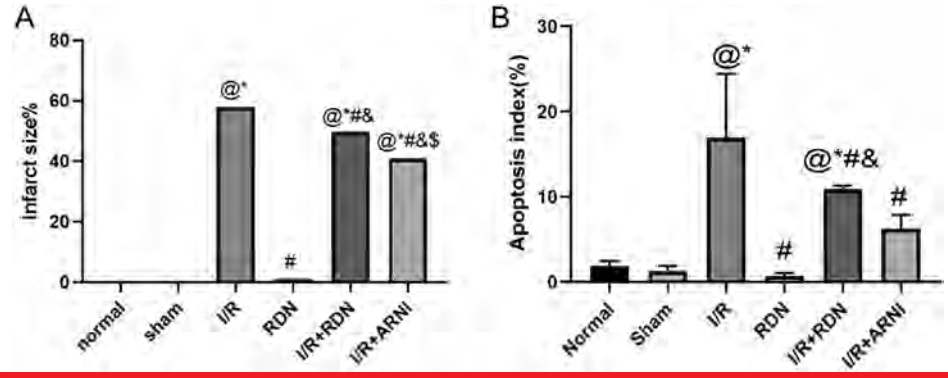
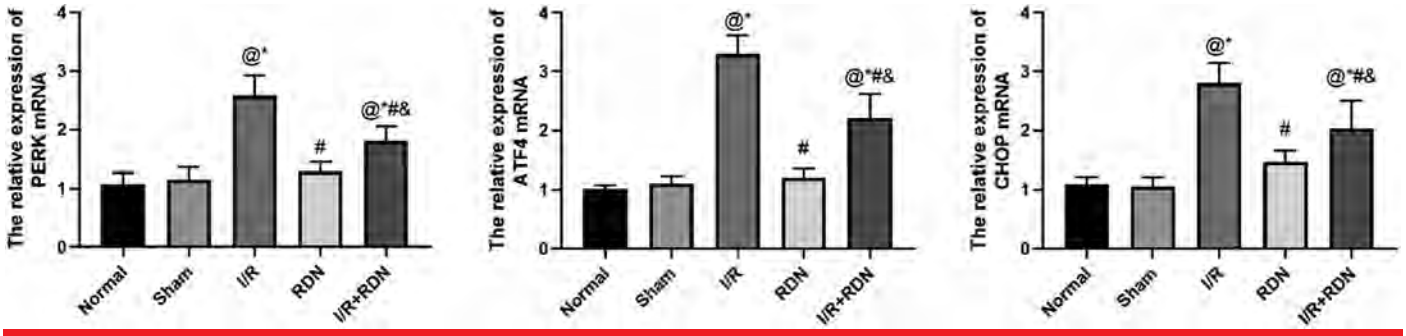
SUPPLEMENTARY MATERIALS



Supplementary Figure 1 . Hormone levels expression in each group. NE, Ang II, and ALD were determined by ELISA in each group. $^{@}P < .05$ vs. normal group, $^{*}P < .05$ vs. sham group, $^{\#}P < .05$ vs. I/R group, $^{\&}P < .05$ vs. RDN group, $^{\$}P < .05$ vs. I/R+RDN group.



Supplementary Figure 2 . Quantitative analysis of the infarct size of TTC staining and the proportion of TUNEL-positive cells. (a) The infarct size in Each group had 0.20% in normal group, 0.40% in sham group, 57.88% in I/R group, 1.1% in RDN group, 49.70% in I/R+RDN group, 40.87% 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, $^{\$}P<0.05$ vs. I/R+RDN group. (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.



Supplementary Figure 3 . Expression of PERK, ATF-4, and CHOP in 5 groups determined by RT-PCR. $^{@}P < .05$ vs. normal group, $^{*}P < .05$ vs. sham group, $^{\#}P < .05$ vs. I/R group, $^{\&}P < .05$ vs. RDN group.

Tukey's multiple comparisons test and *P* value:

Figure 2a:

SBP 1W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	1.333	-25.97 to 28.64	No	ns	>0.9999
Normal vs. I/R	-3.000	-30.30 to 24.30	No	ns	0.9989
Normal vs. RDN	9.667	-17.64 to 36.97	No	ns	0.8338
Normal vs. I/R+RDN	4.333	-22.97 to 31.64	No	ns	0.9936
Normal vs. I/R+ARNI	3.333	-23.97 to 30.64	No	ns	0.9981
Sham vs. I/R	-4.333	-31.64 to 22.97	No	ns	0.9936
Sham vs. RDN	8.333	-18.97 to 35.64	No	ns	0.9006
Sham vs. I/R+RDN	3.000	-24.30 to 30.30	No	ns	0.9989
Sham vs. I/R+ARNI	2.000	-25.30 to 29.30	No	ns	0.9998
I/R vs. RDN	12.67	-14.64 to 39.97	No	ns	0.6374
I/R vs. I/R+RDN	7.333	-19.97 to 34.64	No	ns	0.9386
I/R vs. I/R+ARNI	6.333	-20.97 to 33.64	No	ns	0.9660
RDN vs. I/R+RDN	-5.333	-32.64 to 21.97	No	ns	0.9837
RDN vs. I/R+ARNI	-6.333	-33.64 to 20.97	No	ns	0.9660
I/R+RDN vs. I/R+ARNI	-1.000	-28.30 to 26.30	No	ns	>0.9999

DBP 1W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	4.000	-11.78 to 19.78	No	ns	0.9512
Normal vs. I/R	2.000	-13.78 to 17.78	No	ns	0.9977
Normal vs. RDN	7.667	-8.115 to 23.45	No	ns	0.5952
Normal vs. I/R+RDN	4.000	-11.78 to 19.78	No	ns	0.9512
Normal vs. I/R+ARNI	4.333	-11.45 to 20.11	No	ns	0.9332
Sham vs. I/R	-2.000	-17.78 to 13.78	No	ns	0.9977
Sham vs. RDN	3.667	-12.11 to 19.45	No	ns	0.9658
Sham vs. I/R+RDN	0.000	-15.78 to 15.78	No	ns	>0.9999
Sham vs. I/R+ARNI	0.3333	-15.45 to 16.11	No	ns	>0.9999
I/R vs. RDN	5.667	-10.11 to 21.45	No	ns	0.8260
I/R vs. I/R+RDN	2.000	-13.78 to 17.78	No	ns	0.9977
I/R vs. I/R+ARNI	2.333	-13.45 to 18.11	No	ns	0.9954
RDN vs. I/R+RDN	-3.667	-19.45 to 12.11	No	ns	0.9658
RDN vs. I/R+ARNI	-3.333	-19.11 to 12.45	No	ns	0.9771
I/R+RDN vs. I/R+ARNI	0.3333	-15.45 to 16.11	No	ns	>0.9999

Heart rate 1W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	10.00	-56.05 to 76.05	No	ns	0.9948
Normal vs. I/R	-8.000	-74.05 to 58.05	No	ns	0.9982
Normal vs. RDN	19.00	-47.05 to 85.05	No	ns	0.9202
Normal vs. I/R+RDN	20.00	-46.05 to 86.05	No	ns	0.9034
Normal vs. I/R+ARNI	5.000	-61.05 to 71.05	No	ns	0.9998
Sham vs. I/R	-18.00	-84.05 to 48.05	No	ns	0.9351
Sham vs. RDN	9.000	-57.05 to 75.05	No	ns	0.9968
Sham vs. I/R+RDN	10.00	-56.05 to 76.05	No	ns	0.9948
Sham vs. I/R+ARNI	-5.000	-71.05 to 61.05	No	ns	0.9998
I/R vs. RDN	27.00	-39.05 to 93.05	No	ns	0.7414
I/R vs. I/R+RDN	28.00	-38.05 to 94.05	No	ns	0.7135
I/R vs. I/R+ARNI	13.00	-53.05 to 79.05	No	ns	0.9831
RDN vs. I/R+RDN	1.000	-65.05 to 67.05	No	ns	>0.9999
RDN vs. I/R+ARNI	-14.00	-80.05 to 52.05	No	ns	0.9768
I/R+RDN vs. I/R+ARNI	-15.00	-81.05 to 51.05	No	ns	0.9689

EF 1W

Tukey's multiple comparisons test	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-2.661 to 6.661	No	ns	0.7039
Normal vs. I/R	27.67 to 36.99	Yes	****	<0.0001
Normal vs. RDN	-1.328 to 7.995	No	ns	0.2294
Normal vs. I/R+RDN	27.34 to 36.66	Yes	****	<0.0001
Normal vs. I/R+ARNI	25.34 to 34.66	Yes	****	<0.0001
Sham vs. I/R	25.67 to 34.99	Yes	****	<0.0001
Sham vs. RDN	-3.328 to 5.995	No	ns	0.9219
Sham vs. I/R+RDN	25.34 to 34.66	Yes	****	<0.0001
Sham vs. I/R+ARNI	23.34 to 32.66	Yes	****	<0.0001
I/R vs. RDN	-33.66 to -24.34	Yes	****	<0.0001
I/R vs. I/R+RDN	-4.995 to 4.328	No	ns	0.9999
I/R vs. I/R+ARNI	-6.995 to 2.328	No	ns	0.5668
RDN vs. I/R+RDN	24.01 to 33.33	Yes	****	<0.0001
RDN vs. I/R+ARNI	22.01 to 31.33	Yes	****	<0.0001
I/R+RDN vs. I/R+ARNI	-6.661 to 2.661	No	ns	0.7039

FS 1W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	2.000	-1.879 to 5.879	No	ns	0.5380
Normal vs. I/R	22.33	18.45 to 26.21	Yes	****	<0.0001
Normal vs. RDN	2.667	-1.212 to 6.545	No	ns	0.2619
Normal vs. I/R+RDN	21.67	17.79 to 25.55	Yes	****	<0.0001
Normal vs. I/R+ARNI	20.67	16.79 to 24.55	Yes	****	<0.0001
Sham vs. I/R	20.33	16.45 to 24.21	Yes	****	<0.0001
Sham vs. RDN	0.6667	-3.212 to 4.545	No	ns	0.9908
Sham vs. I/R+RDN	19.67	15.79 to 23.55	Yes	****	<0.0001
Sham vs. I/R+ARNI	18.67	14.79 to 22.55	Yes	****	<0.0001
I/R vs. RDN	-19.67	-23.55 to -15.79	Yes	****	<0.0001
I/R vs. I/R+RDN	-0.6667	-4.545 to 3.212	No	ns	0.9908
I/R vs. I/R+ARNI	-1.667	-5.545 to 2.212	No	ns	0.7027
RDN vs. I/R+RDN	19.00	15.12 to 22.88	Yes	****	<0.0001
RDN vs. I/R+ARNI	18.00	14.12 to 21.88	Yes	****	<0.0001
I/R+RDN vs. I/R+ARNI	-1.000	-4.879 to 2.879	No	ns	0.9478

LVSD 1W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	0.07667	-0.1059 to 0.2593	No	ns	0.7211
Normal vs. I/R	-2.163	-2.346 to -1.981	Yes	****	<0.0001
Normal vs. RDN	0.09667	-0.08594 to 0.2793	No	ns	0.5122
Normal vs. I/R+RDN	-2.160	-2.343 to -1.977	Yes	****	<0.0001
Normal vs. I/R+ARNI	-2.180	-2.363 to -1.997	Yes	****	<0.0001
Sham vs. I/R	-2.240	-2.423 to -2.057	Yes	****	<0.0001
Sham vs. RDN	0.02000	-0.1626 to 0.2026	No	ns	0.9989
Sham vs. I/R+RDN	-2.237	-2.419 to -2.054	Yes	****	<0.0001
Sham vs. I/R+ARNI	-2.257	-2.439 to -2.074	Yes	****	<0.0001
I/R vs. RDN	2.260	2.077 to 2.443	Yes	****	<0.0001
I/R vs. I/R+RDN	0.003333	-0.1793 to 0.1859	No	ns	>0.9999
I/R vs. I/R+ARNI	-0.01667	-0.1993 to 0.1659	No	ns	0.9995
RDN vs. I/R+RDN	-2.257	-2.439 to -2.074	Yes	****	<0.0001
RDN vs. I/R+ARNI	-2.277	-2.459 to -2.094	Yes	****	<0.0001
I/R+RDN vs. I/R+ARNI	-0.02000	-0.2026 to 0.1626	No	ns	0.9989

LVDd

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-0.04667	-0.3969 to 0.3036	No	ns	0.9971
Normal vs. I/R	-0.5467	-0.8969 to -0.1964	Yes	**	0.0022
Normal vs. RDN	-0.03000	-0.3803 to 0.3203	No	ns	0.9997
Normal vs. I/R+RDN	-0.5333	-0.8836 to -0.1831	Yes	**	0.0027
Normal vs. I/R+ARNI	-0.5467	-0.8969 to -0.1964	Yes	**	0.0022
Sham vs. I/R	-0.5000	-0.8503 to -0.1497	Yes	**	0.0045
Sham vs. RDN	0.01667	-0.3336 to 0.3669	No	ns	>0.9999
Sham vs. I/R+RDN	-0.4867	-0.8369 to -0.1364	Yes	**	0.0056
Sham vs. I/R+ARNI	-0.5000	-0.8503 to -0.1497	Yes	**	0.0045
I/R vs. RDN	0.5167	0.1664 to 0.8669	Yes	**	0.0035
I/R vs. I/R+RDN	0.01333	-0.3369 to 0.3636	No	ns	>0.9999
I/R vs. I/R+ARNI	0.000	-0.3503 to 0.3503	No	ns	>0.9999
RDN vs. I/R+RDN	-0.5033	-0.8536 to -0.1531	Yes	**	0.0043
RDN vs. I/R+ARNI	-0.5167	-0.8669 to -0.1664	Yes	**	0.0035
I/R+RDN vs. I/R+ARNI	-0.01333	-0.3636 to 0.3369	No	ns	>0.9999

Figure 2b:

SBP 3W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	1.000	-26.77 to 28.77	No	ns	>0.9999
Normal vs. I/R	8.333	-19.44 to 36.11	No	ns	0.9066
Normal vs. RDN	6.000	-21.77 to 33.77	No	ns	0.9748
Normal vs. I/R+RDN	1.000	-26.77 to 28.77	No	ns	>0.9999
Normal vs. I/R+ARNI	5.000	-22.77 to 32.77	No	ns	0.9886
Sham vs. I/R	7.333	-20.44 to 35.11	No	ns	0.9426
Sham vs. RDN	5.000	-22.77 to 32.77	No	ns	0.9886
Sham vs. I/R+RDN	0.000	-27.77 to 27.77	No	ns	>0.9999
Sham vs. I/R+ARNI	4.000	-23.77 to 31.77	No	ns	0.9959
I/R vs. RDN	-2.333	-30.11 to 25.44	No	ns	0.9997
I/R vs. I/R+RDN	-7.333	-35.11 to 20.44	No	ns	0.9426
I/R vs. I/R+ARNI	-3.333	-31.11 to 24.44	No	ns	0.9983
RDN vs. I/R+RDN	-5.000	-32.77 to 22.77	No	ns	0.9886
RDN vs. I/R+ARNI	-1.000	-28.77 to 26.77	No	ns	>0.9999
I/R+RDN vs. I/R+ARNI	4.000	-23.77 to 31.77	No	ns	0.9959

DBP 3W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-1.333	-20.57 to 17.91	No	ns	0.9999
Normal vs. I/R	3.333	-15.91 to 22.57	No	ns	0.9904
Normal vs. RDN	3.000	-16.24 to 22.24	No	ns	0.9941
Normal vs. I/R+RDN	-1.333	-20.57 to 17.91	No	ns	0.9999
Normal vs. I/R+ARNI	2.333	-16.91 to 21.57	No	ns	0.9982
Sham vs. I/R	4.667	-14.57 to 23.91	No	ns	0.9592
Sham vs. RDN	4.333	-14.91 to 23.57	No	ns	0.9700
Sham vs. I/R+RDN	0.000	-19.24 to 19.24	No	ns	>0.9999
Sham vs. I/R+ARNI	3.667	-15.57 to 22.91	No	ns	0.9854
I/R vs. RDN	-0.3333	-19.57 to 18.91	No	ns	>0.9999
I/R vs. I/R+RDN	-4.667	-23.91 to 14.57	No	ns	0.9592
I/R vs. I/R+ARNI	-1.000	-20.24 to 18.24	No	ns	>0.9999
RDN vs. I/R+RDN	-4.333	-23.57 to 14.91	No	ns	0.9700
RDN vs. I/R+ARNI	-0.6667	-19.91 to 18.57	No	ns	>0.9999
I/R+RDN vs. I/R+ARNI	3.667	-15.57 to 22.91	No	ns	0.9854

HR 3W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	8.667	-35.93 to 53.27	No	ns	0.9840
Normal vs. I/R	56.00	11.40 to 100.6	Yes	*	0.0118
Normal vs. RDN	10.33	-34.27 to 54.93	No	ns	0.9662
Normal vs. I/R+RDN	22.00	-22.60 to 66.60	No	ns	0.5807
Normal vs. I/R+ARNI	20.00	-24.60 to 64.60	No	ns	0.6672
Sham vs. I/R	47.33	2.735 to 91.93	Yes	*	0.0354
Sham vs. RDN	1.667	-42.93 to 46.27	No	ns	>0.9999
Sham vs. I/R+RDN	13.33	-31.27 to 57.93	No	ns	0.9079
Sham vs. I/R+ARNI	11.33	-33.27 to 55.93	No	ns	0.9507
I/R vs. RDN	-45.67	-90.27 to -1.068	Yes	*	0.0437
I/R vs. I/R+RDN	-34.00	-78.60 to 10.60	No	ns	0.1812
I/R vs. I/R+ARNI	-36.00	-80.60 to 8.599	No	ns	0.1436
RDN vs. I/R+RDN	11.67	-32.93 to 56.27	No	ns	0.9447
RDN vs. I/R+ARNI	9.667	-34.93 to 54.27	No	ns	0.9744
I/R+RDN vs. I/R+ARNI	-2.000	-46.60 to 42.60	No	ns	>0.9999

EF 3W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	1.333	-4.901 to 7.567	No	ns	0.9758
Normal vs. I/R	31.67	25.43 to 37.90	Yes	****	<0.0001
Normal vs. RDN	2.667	-3.567 to 8.901	No	ns	0.7064
Normal vs. I/R+RDN	21.00	14.77 to 27.23	Yes	****	<0.0001
Normal vs. I/R+ARNI	11.67	5.433 to 17.90	Yes	***	0.0004
Sham vs. I/R	30.33	24.10 to 36.57	Yes	****	<0.0001
Sham vs. RDN	1.333	-4.901 to 7.567	No	ns	0.9758
Sham vs. I/R+RDN	19.67	13.43 to 25.90	Yes	****	<0.0001
Sham vs. I/R+ARNI	10.33	4.099 to 16.57	Yes	**	0.0013
I/R vs. RDN	-29.00	-35.23 to -22.77	Yes	****	<0.0001
I/R vs. I/R+RDN	-10.67	-16.90 to -4.433	Yes	***	0.0010
I/R vs. I/R+ARNI	-20.00	-26.23 to -13.77	Yes	****	<0.0001
RDN vs. I/R+RDN	18.33	12.10 to 24.57	Yes	****	<0.0001
RDN vs. I/R+ARNI	9.000	2.766 to 15.23	Yes	**	0.0041
I/R+RDN vs. I/R+ARNI	-9.333	-15.57 to -3.099	Yes	**	0.0031

FS 3W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	1.000	-2.296 to 4.296	No	ns	0.9028
Normal vs. I/R	22.00	18.70 to 25.30	Yes	****	<0.0001
Normal vs. RDN	1.333	-1.963 to 4.629	No	ns	0.7491
Normal vs. I/R+RDN	10.00	6.704 to 13.30	Yes	****	<0.0001
Normal vs. I/R+ARNI	7.667	4.371 to 10.96	Yes	****	<0.0001
Sham vs. I/R	21.00	17.70 to 24.30	Yes	****	<0.0001
Sham vs. RDN	0.3333	-2.963 to 3.629	No	ns	0.9992
Sham vs. I/R+RDN	9.000	5.704 to 12.30	Yes	****	<0.0001
Sham vs. I/R+ARNI	6.667	3.371 to 9.963	Yes	***	0.0002
I/R vs. RDN	-20.67	-23.96 to -17.37	Yes	****	<0.0001
I/R vs. I/R+RDN	-12.00	-15.30 to -8.704	Yes	****	<0.0001
I/R vs. I/R+ARNI	-14.33	-17.63 to -11.04	Yes	****	<0.0001
RDN vs. I/R+RDN	8.667	5.371 to 11.96	Yes	****	<0.0001
RDN vs. I/R+ARNI	6.333	3.037 to 9.629	Yes	***	0.0003
I/R+RDN vs. I/R+ARNI	-2.333	-5.629 to 0.9628	No	ns	0.2375

LVSD 3W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	0.07667	-0.1531 to 0.3064	No	ns	0.8637
Normal vs. I/R	-3.607	-3.836 to -3.377	Yes	****	<0.0001
Normal vs. RDN	0.09667	-0.1331 to 0.3264	No	ns	0.7194
Normal vs. I/R+RDN	-2.300	-2.530 to -2.070	Yes	****	<0.0001
Normal vs. I/R+ARNI	-2.123	-2.353 to -1.894	Yes	****	<0.0001
Sham vs. I/R	-3.683	-3.913 to -3.454	Yes	****	<0.0001
Sham vs. RDN	0.02000	-0.2097 to 0.2497	No	ns	0.9996
Sham vs. I/R+RDN	-2.377	-2.606 to -2.147	Yes	****	<0.0001
Sham vs. I/R+ARNI	-2.200	-2.430 to -1.970	Yes	****	<0.0001
I/R vs. RDN	3.703	3.474 to 3.933	Yes	****	<0.0001
I/R vs. I/R+RDN	1.307	1.077 to 1.536	Yes	****	<0.0001
I/R vs. I/R+ARNI	1.483	1.254 to 1.713	Yes	****	<0.0001
RDN vs. I/R+RDN	-2.397	-2.626 to -2.167	Yes	****	<0.0001
RDN vs. I/R+ARNI	-2.220	-2.450 to -1.990	Yes	****	<0.0001
I/R+RDN vs. I/R+ARNI	0.1767	-0.05306 to 0.4064	No	ns	0.1751

LVDd 3W

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-0.04667	-0.4397 to 0.3464	No	ns	0.9983
Normal vs. I/R	-2.363	-2.756 to -1.970	Yes	****	<0.0001
Normal vs. RDN	-0.03000	-0.4230 to 0.3630	No	ns	0.9998
Normal vs. I/R+RDN	-0.5333	-0.9264 to -0.1403	Yes	**	0.0067
Normal vs. I/R+ARNI	-0.2167	-0.6097 to 0.1764	No	ns	0.4720
Sham vs. I/R	-2.317	-2.710 to -1.924	Yes	****	<0.0001
Sham vs. RDN	0.01667	-0.3764 to 0.4097	No	ns	>0.9999
Sham vs. I/R+RDN	-0.4867	-0.8797 to -0.09362	Yes	*	0.0130
Sham vs. I/R+ARNI	-0.1700	-0.5630 to 0.2230	No	ns	0.6974
I/R vs. RDN	2.333	1.940 to 2.726	Yes	****	<0.0001
I/R vs. I/R+RDN	1.830	1.437 to 2.223	Yes	****	<0.0001
I/R vs. I/R+ARNI	2.147	1.754 to 2.540	Yes	****	<0.0001
RDN vs. I/R+RDN	-0.5033	-0.8964 to -0.1103	Yes	*	0.0102
RDN vs. I/R+ARNI	-0.1867	-0.5797 to 0.2064	No	ns	0.6162
I/R+RDN vs. I/R+ARNI	0.3167	-0.07638 to 0.7097	No	ns	0.1448

Figure 4B

Bax

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-0.003333	-0.2890 to 0.2823	No	ns	>0.9999
Normal vs. I/R	-0.3643	-0.6500 to -0.07872	Yes	*	0.0105
Normal vs. RDN	-0.01700	-0.3026 to 0.2686	No	ns	>0.9999
Normal vs. I/R+RDN	-0.2140	-0.4996 to 0.07162	No	ns	0.1936
Normal vs. I/R+ARNI	-0.1503	-0.4360 to 0.1353	No	ns	0.5179
Sham vs. I/R	-0.3610	-0.6466 to -0.07538	Yes	*	0.0112
Sham vs. RDN	-0.01367	-0.2993 to 0.2720	No	ns	>0.9999
Sham vs. I/R+RDN	-0.2107	-0.4963 to 0.07495	No	ns	0.2052
Sham vs. I/R+ARNI	-0.1470	-0.4326 to 0.1386	No	ns	0.5399
I/R vs. RDN	0.3473	0.06172 to 0.6330	Yes	*	0.0147
I/R vs. I/R+RDN	0.1503	-0.1353 to 0.4360	No	ns	0.5179
I/R vs. I/R+ARNI	0.2140	-0.07162 to 0.4996	No	ns	0.1936
RDN vs. I/R+RDN	-0.1970	-0.4826 to 0.08862	No	ns	0.2592
RDN vs. I/R+ARNI	-0.1333	-0.4190 to 0.1523	No	ns	0.6318
I/R+RDN vs. I/R+ARNI	0.06367	-0.2220 to 0.3493	No	ns	0.9712

Figure 4C

Bcl2

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-0.0006667	-0.1603 to 0.1590	No	ns	>0.9999
Normal vs. I/R	0.4433	0.2837 to 0.6030	Yes	****	<0.0001
Normal vs. RDN	0.04767	-0.1120 to 0.2073	No	ns	0.9083
Normal vs. I/R+RDN	0.2483	0.08867 to 0.4080	Yes	**	0.0023
Normal vs. I/R+ARNI	0.2060	0.04634 to 0.3657	Yes	**	0.0097
Sham vs. I/R	0.4440	0.2843 to 0.6037	Yes	****	<0.0001
Sham vs. RDN	0.04833	-0.1113 to 0.2080	No	ns	0.9035
Sham vs. I/R+RDN	0.2490	0.08934 to 0.4087	Yes	**	0.0022
Sham vs. I/R+ARNI	0.2067	0.04701 to 0.3663	Yes	**	0.0095
I/R vs. RDN	-0.3957	-0.5553 to -0.2360	Yes	****	<0.0001
I/R vs. I/R+RDN	-0.1950	-0.3547 to -0.03534	Yes	*	0.0143
I/R vs. I/R+ARNI	-0.2373	-0.3970 to -0.07767	Yes	**	0.0033
RDN vs. I/R+RDN	0.2007	0.04101 to 0.3603	Yes	*	0.0117
RDN vs. I/R+ARNI	0.1583	-0.001328 to 0.3180	No	ns	0.0524
I/R+RDN vs. I/R+ARNI	-0.04233	-0.2020 to 0.1173	No	ns	0.9417

Figure 4D

Caspase-3

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	0.000	-0.08376 to 0.08376	No	ns	>0.9999
Normal vs. I/R	-0.1283	-0.2121 to -0.04458	Yes	**	0.0026
Normal vs. RDN	-0.005000	-0.08876 to 0.07876	No	ns	>0.9999
Normal vs. I/R+RDN	-0.07667	-0.1604 to 0.007090	No	ns	0.0802
Normal vs. I/R+ARNI	-0.06233	-0.1461 to 0.02142	No	ns	0.1985
Sham vs. I/R	-0.1283	-0.2121 to -0.04458	Yes	**	0.0026
Sham vs. RDN	-0.005000	-0.08876 to 0.07876	No	ns	>0.9999
Sham vs. I/R+RDN	-0.07667	-0.1604 to 0.007090	No	ns	0.0802
Sham vs. I/R+ARNI	-0.06233	-0.1461 to 0.02142	No	ns	0.1985
I/R vs. RDN	0.1233	0.03958 to 0.2071	Yes	**	0.0035
I/R vs. I/R+RDN	0.05167	-0.03209 to 0.1354	No	ns	0.3611
I/R vs. I/R+ARNI	0.06600	-0.01776 to 0.1498	No	ns	0.1588
RDN vs. I/R+RDN	-0.07167	-0.1554 to 0.01209	No	ns	0.1110
RDN vs. I/R+ARNI	-0.05733	-0.1411 to 0.02642	No	ns	0.2657
I/R+RDN vs. I/R+ARNI	0.01433	-0.06942 to 0.09809	No	ns	0.9909

Figure 5B

FERK

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-0.005000	-0.1304 to 0.1204	No	ns	>0.9999
Normal vs. I/R	-0.3087	-0.4341 to -0.1833	Yes	****	<0.0001
Normal vs. RDN	-0.05000	-0.1754 to 0.07539	No	ns	0.6904
Normal vs. I/R+RDN	-0.1980	-0.3234 to -0.07261	Yes	**	0.0029
Sham vs. I/R	-0.3037	-0.4291 to -0.1783	Yes	****	<0.0001
Sham vs. RDN	-0.04500	-0.1704 to 0.08039	No	ns	0.7617
Sham vs. I/R+RDN	-0.1930	-0.3184 to -0.06761	Yes	**	0.0035
I/R vs. RDN	0.2587	0.1333 to 0.3841	Yes	***	0.0004
I/R vs. I/R+RDN	0.1107	-0.01473 to 0.2361	No	ns	0.0908
RDN vs. I/R+RDN	-0.1480	-0.2734 to -0.02261	Yes	*	0.0199

Figure 5C

CHOP

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-0.006333	-0.1303 to 0.1176	No	ns	0.9998
Normal vs. I/R	-0.4767	-0.6006 to -0.3527	Yes	****	<0.0001
Normal vs. RDN	-0.03300	-0.1569 to 0.09093	No	ns	0.8993
Normal vs. I/R+RDN	-0.2607	-0.3846 to -0.1367	Yes	***	0.0003
Sham vs. I/R	-0.4703	-0.5943 to -0.3464	Yes	****	<0.0001
Sham vs. RDN	-0.02667	-0.1506 to 0.09726	No	ns	0.9499
Sham vs. I/R+RDN	-0.2543	-0.3783 to -0.1304	Yes	***	0.0004
I/R vs. RDN	0.4437	0.3197 to 0.5676	Yes	****	<0.0001
I/R vs. I/R+RDN	0.2160	0.09207 to 0.3399	Yes	**	0.0014
RDN vs. I/R+RDN	-0.2277	-0.3516 to -0.1037	Yes	***	0.0009

Figure 5D

ATF4

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	0.04767	-0.1406 to 0.2359	No	ns	0.9141
Normal vs. I/R	-0.3503	-0.5386 to -0.1621	Yes	***	0.0008
Normal vs. RDN	-0.005333	-0.1936 to 0.1829	No	ns	>0.9999
Normal vs. I/R+RDN	-0.1663	-0.3546 to 0.02189	No	ns	0.0903
Sham vs. I/R	-0.3980	-0.5862 to -0.2098	Yes	***	0.0003
Sham vs. RDN	-0.05300	-0.2412 to 0.1352	No	ns	0.8802
Sham vs. I/R+RDN	-0.2140	-0.4022 to -0.02578	Yes	*	0.0249
I/R vs. RDN	0.3450	0.1568 to 0.5332	Yes	***	0.0009
I/R vs. I/R+RDN	0.1840	-0.004223 to 0.3722	No	ns	0.0561
RDN vs. I/R+RDN	-0.1610	-0.3492 to 0.02722	No	ns	0.1040

Supplementary Figure 1

NE

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-0.04367	-0.3330 to 0.2457	No	ns	0.9949
Normal vs. I/R	-1.449	-1.738 to -1.160	Yes	****	<0.0001
Normal vs. RDN	0.01433	-0.2750 to 0.3037	No	ns	>0.9999
Normal vs. I/R+RDN	-0.5297	-0.8190 to -0.2403	Yes	***	0.0005
Normal vs. I/R+ARNI	-1.016	-1.305 to -0.7267	Yes	****	<0.0001
Sham vs. I/R	-1.405	-1.695 to -1.116	Yes	****	<0.0001
Sham vs. RDN	0.05800	-0.2313 to 0.3473	No	ns	0.9817
Sham vs. I/R+RDN	-0.4860	-0.7753 to -0.1967	Yes	**	0.0012
Sham vs. I/R+ARNI	-0.9723	-1.262 to -0.6830	Yes	****	<0.0001
I/R vs. RDN	1.463	1.174 to 1.753	Yes	****	<0.0001
I/R vs. I/R+RDN	0.9193	0.6300 to 1.209	Yes	****	<0.0001
I/R vs. I/R+ARNI	0.4330	0.1437 to 0.7223	Yes	**	0.0031
RDN vs. I/R+RDN	-0.5440	-0.8333 to -0.2547	Yes	***	0.0004
RDN vs. I/R+ARNI	-1.030	-1.320 to -0.7410	Yes	****	<0.0001
I/R+RDN vs. I/R+ARNI	-0.4863	-0.7757 to -0.1970	Yes	**	0.0012

Ang II

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-7.879	-40.10 to 24.34	No	ns	0.9578
Normal vs. I/R	-142.7	-174.9 to -110.5	Yes	****	<0.0001
Normal vs. RDN	-9.510	-41.73 to 22.71	No	ns	0.9121
Normal vs. I/R+RDN	-98.93	-131.1 to -66.72	Yes	****	<0.0001
Normal vs. I/R+ARNI	-65.84	-98.06 to -33.63	Yes	***	0.0002
Sham vs. I/R	-134.8	-167.0 to -102.6	Yes	****	<0.0001
Sham vs. RDN	-1.631	-33.85 to 30.59	No	ns	>0.9999
Sham vs. I/R+RDN	-91.05	-123.3 to -58.84	Yes	****	<0.0001
Sham vs. I/R+ARNI	-57.96	-90.18 to -25.75	Yes	***	0.0006
I/R vs. RDN	133.2	101.0 to 165.4	Yes	****	<0.0001
I/R vs. I/R+RDN	43.77	11.56 to 75.99	Yes	**	0.0066
I/R vs. I/R+ARNI	76.87	44.65 to 109.1	Yes	****	<0.0001
RDN vs. I/R+RDN	-89.42	-121.6 to -57.21	Yes	****	<0.0001
RDN vs. I/R+ARNI	-56.33	-88.55 to -24.12	Yes	***	0.0008
I/R+RDN vs. I/R+ARNI	33.09	0.8761 to 65.31	Yes	*	0.0429

ALD

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-19.68	-138.9 to 99.50	No	ns	0.9923
Normal vs. I/R	-493.0	-612.2 to -373.8	Yes	****	<0.0001
Normal vs. RDN	-5.354	-124.5 to 113.8	No	ns	>0.9999
Normal vs. I/R+RDN	-254.1	-373.3 to -134.9	Yes	***	0.0001
Normal vs. I/R+ARNI	-172.6	-291.8 to -53.41	Yes	**	0.0040
Sham vs. I/R	-473.3	-592.5 to -354.1	Yes	****	<0.0001
Sham vs. RDN	14.33	-104.9 to 133.5	No	ns	0.9982
Sham vs. I/R+RDN	-234.4	-353.6 to -115.2	Yes	***	0.0003
Sham vs. I/R+ARNI	-152.9	-272.1 to -33.73	Yes	*	0.0101
I/R vs. RDN	487.6	368.5 to 606.8	Yes	****	<0.0001
I/R vs. I/R+RDN	238.9	119.7 to 358.1	Yes	***	0.0002
I/R vs. I/R+ARNI	320.4	201.2 to 439.6	Yes	****	<0.0001
RDN vs. I/R+RDN	-248.7	-367.9 to -129.5	Yes	***	0.0002
RDN vs. I/R+ARNI	-167.2	-286.4 to -48.05	Yes	**	0.0052
I/R+RDN vs. I/R+ARNI	81.49	-37.69 to 200.7	No	ns	0.2666

Supplementary Figure 2

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	0.5867	-8.031 to 9.204	No	ns	0.9999
Normal vs. I/R	-15.07	-23.69 to -6.456	Yes	***	0.0008
Normal vs. RDN	0.6733	-7.944 to 9.291	No	ns	0.9998
Normal vs. I/R+RDN	-8.990	-17.61 to -0.3727	Yes	*	0.0392
Normal vs. I/R+ARNI	-4.340	-12.96 to 4.277	No	ns	0.5609
Sham vs. I/R	-15.66	-24.28 to -7.043	Yes	***	0.0006
Sham vs. RDN	0.08667	-8.531 to 8.704	No	ns	>0.9999
Sham vs. I/R+RDN	-9.577	-18.19 to -0.9593	Yes	*	0.0266
Sham vs. I/R+ARNI	-4.927	-13.54 to 3.691	No	ns	0.4357
I/R vs. RDN	15.75	7.129 to 24.36	Yes	***	0.0006
I/R vs. I/R+RDN	6.083	-2.534 to 14.70	No	ns	0.2398
I/R vs. I/R+ARNI	10.73	2.116 to 19.35	Yes	*	0.0124
RDN vs. I/R+RDN	-9.663	-18.28 to -1.046	Yes	*	0.0252
RDN vs. I/R+ARNI	-5.013	-13.63 to 3.604	No	ns	0.4184
I/R+RDN vs. I/R+ARNI	4.650	-3.967 to 13.27	No	ns	0.4932

Supplementary Figure 3

PERK

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-0.07667	-0.7169 to 0.5636	No	ns	0.9941
Normal vs. I/R	-1.504	-2.145 to -0.8641	Yes	***	0.0001
Normal vs. RDN	-0.2083	-0.8486 to 0.4319	No	ns	0.8171
Normal vs. I/R+RDN	-0.7287	-1.369 to -0.08845	Yes	*	0.0247
Sham vs. I/R	-1.428	-2.068 to -0.7874	Yes	***	0.0002
Sham vs. RDN	-0.1317	-0.7719 to 0.5086	No	ns	0.9571
Sham vs. I/R+RDN	-0.6520	-1.292 to -0.01178	Yes	*	0.0455
I/R vs. RDN	1.296	0.6558 to 1.936	Yes	***	0.0004
I/R vs. I/R+RDN	0.7757	0.1354 to 1.416	Yes	*	0.0170
RDN vs. I/R+RDN	-0.5203	-1.161 to 0.1199	No	ns	0.1285

ATF4

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	-0.09467	-0.7682 to 0.5788	No	ns	0.9891
Normal vs. I/R	-2.283	-2.957 to -1.610	Yes	****	<0.0001
Normal vs. RDN	-0.1897	-0.8632 to 0.4838	No	ns	0.8802
Normal vs. I/R+RDN	-1.198	-1.872 to -0.5245	Yes	**	0.0012
Sham vs. I/R	-2.189	-2.862 to -1.515	Yes	****	<0.0001
Sham vs. RDN	-0.09500	-0.7685 to 0.5785	No	ns	0.9890
Sham vs. I/R+RDN	-1.103	-1.777 to -0.4298	Yes	**	0.0022
I/R vs. RDN	2.094	1.420 to 2.767	Yes	****	<0.0001
I/R vs. I/R+RDN	1.085	0.4118 to 1.759	Yes	**	0.0025
RDN vs. I/R+RDN	-1.008	-1.682 to -0.3348	Yes	**	0.0042

CHOP

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Normal vs. Sham	0.03267	-0.7441 to 0.8094	No	ns	>0.9999
Normal vs. I/R	-1.715	-2.492 to -0.9383	Yes	***	0.0002
Normal vs. RDN	-0.3740	-1.151 to 0.4027	No	ns	0.5373
Normal vs. I/R+RDN	-0.9410	-1.718 to -0.1643	Yes	*	0.0170
Sham vs. I/R	-1.748	-2.524 to -0.9709	Yes	***	0.0002
Sham vs. RDN	-0.4067	-1.183 to 0.3701	No	ns	0.4630
Sham vs. I/R+RDN	-0.9737	-1.750 to -0.1969	Yes	*	0.0138
I/R vs. RDN	1.341	0.5643 to 2.118	Yes	**	0.0015
I/R vs. I/R+RDN	0.7740	-0.002748 to 1.551	No	ns	0.0509
RDN vs. I/R+RDN	-0.5670	-1.344 to 0.2097	No	ns	0.1917