Effects of miR-92a-3P, Oxidative Stress, and the MAPK/NF-κB Pathway on Catheter-Related Thrombosis Prevention After Resistance Exercise

Cui Wen
First Affiliated Hospital of GuangXi Medical University
Yanping Ying (yanpingying0116@126.com)
First Affiliated Hospital of GuangXi Medical University
Huihan Zhao
First Affiliated Hospital of GuangXi Medical University
Qingjuan Jiang
First Affiliated Hospital of GuangXi Medical University
Xiao Gan
First Affiliated Hospital of GuangXi Medical University
Yan Wei
First Affiliated Hospital of GuangXi Medical University
Jiani Wei
First Affiliated Hospital of GuangXi Medical University
Xinxin Huang
First Affiliated Hospital of GuangXi Medical University

Research Article

Keywords: catheter-related thrombosis, resistance exercise intervention, miR-92A-3P, oxidative stress, MAPK/NF-κB pathway

Posted Date: January 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-137539/v1

Effects of miR-92a-3P, Oxidative Stress, and the MAPK/NF-κB Pathway on Catheter-Related Thrombosis Prevention after Resistance Exercise

Cui Wen, Yanping Ying*, Huihan Zhao ,Qingjuan Jiang, Xiao Gan, Yan Wei, Jiani Wei, Xinxin Huang

***Corresponding Author: Correspondence should be addressed to Yanping Ying; yanpingying0116@126.com**

Department of Nursing, The First Affiliated Hospital of Guangxi Medical University, No. 6, ShuangyongRoad, 530021 Nanning, Guangxi, China

Abstract

Background： MiR-92a-3p and oxidative stress are associated with catheter-related thrombosis (CRT). As a kind of physical intervention, resistance exercise can effectively promote blood circulation. In this study, we investigated the roles of miR-92a-3p, oxidative stress, and the P38 mitogen-activated protein kinase/nuclear factor-κB (MAPK/NF-κB) pathway on CRT during resistance exercise.

Methods： The rat CRT model was used for resistance exercise intervention. Moreover, pathological changes from the right jugular vein to the right auricle were observed under an electron microscope. In addition, reactive oxygen species (ROS) production, malondialdehyde (MDA) activity, and heme oxygenase (HO-1) level in rat serum were detected via ELISA. Furthermore, expression levels of miR-92A-3p and HO-1 in the vascular tissues of the rats were detected via real-time quantitative PCR. Additionally, expression levels of HO-1, NF-κB P65, p38MAPK, and IκBa in the venous tissues of the rats were analyzed by Western blot analysis.

Results: Thrombosis incidence rate in CRT+RE group was lower than that in CRT group. In the thrombosis model, markers related to oxidative stress and miR-92a-3p increased. After administering the resistance exercise intervention, ROS production and MDA activity significantly decreased, the expression level of HO-1 increased, and the expression level of miR-92A-3p in the vascular tissues significantly decreased. The levels of p38MAPK and NF-κB p65 significantly decreased but that of IκBa significantly increased.

Conclusion: Resistance exercise intervention downregulated miR-92a-3p expression,

repaired oxidative stress injury, and prevented CRT formation.

Keywords: catheter-related thrombosis, resistance exercise intervention, miR-92A-3P, oxidative stress, MAPK/NF-κB pathway

Introduction

Central venous catheters (CVCs) are extensively applied in medical and health institutions. CVCs can offer a safe and reliable venous access to tumor chemotherapy, parenteral nutrition, long-term infusion, and treatment of critically ill patients [1]. After intravenous catheterization, the catheter punctures the vascular wall, resulting in endothelial cell injury and hemodynamic changes, thereby further promoting blood hypercoagulability [2]. Hence, catheter-related complications are inevitable, among which catheter-related thrombosis (CRT) is the most severe, resulting in pulmonary embolism, recurrent deep vein thrombosis (DVT), post-thrombotic syndrome, and sepsis [3]. Recent studies noted that 16%–18% of patients with CVCs have evidence of CRT as revealed by ultrasound or intravenous screening [4]. Moreover, incidence of asymptomatic CRT is reportedly as high as 68% [5]. CRT cannot be detected without ultrasonic evaluation and testing, and compounding this problem is the fact that only 1%–5% of patients are symptomatic [6]. Therefore, CRT can be characterized as a "high risk, common" disease. CRT can increase pain sensitivity, leading to catheter dysfunction, increased risks of infection, and central venous stenosis; moreover, it increases the length of hospital stay and medical costs [7]. Therefore, CRT prevention is necessary.

The Infusion Nurse Society, following the amendments of the Infusion Practice Standard of 2016, has encouraged patients undergoing infusion to engage in physical activity and exercise as early as possible to prevent CRT formation [8]. Colwell et al. [9] found no statistically significant differences between anticoagulant drugs and physical exercise in terms of reducing DVT incidence. Moreover, compared with anticoagulant drugs, physical activity can effectively minimize or eliminate the risk of bleeding. Early resistance exercises can increase muscle strength and enhance subendocardial blood perfusion by increasing cardiac pressure load to achieve the optimal balance between cardiovascular oxygen supply and demand and improve cardiovascular functions [10].

MiRNAs are a class of endogenous noncoding small RNA molecules that play an important regulatory role in angiogenesis [11]. Members of the miR-17-92 cluster have a considerable influence on the cardiovascular system. Specifically, miR-92a regulates vascular dynamic balance by upregulating the expression of endothelial proinflammatory factors [12, 13]. Micro-92a-3p is reported to be involved in regulating vascular system dysfunctions and mediating blood flow shear stress, and its expression is upregulated in a rat model of deep vein thrombosis [14, 15].

When vessel wall is punctured by a catheter, oxidative stress damaged vascular endothelial cells, leading to ROS and reactive nitrogen over-production and eventually disrupts the redox balance [16]. A high ROS content can increase the level of malondialdehyde (MDA), further causing oxidative stress-induced tissue damage, increasing membrane permeability, and rupturing the double-layered structure of the cell membrane [17]. HO-1 (HMOX1), an antioxidant enzyme found in vascular endothelial cells and smooth muscle cells, can protect damaged blood vessels via inhibiting the proliferation of vascular smooth muscle cells [18].

Pro-apoptotic pathways (i.e., mitogen-activated protein kinases [MAPKs]) and inflammatory response pathways (i.e., NF-κB) are important in regulating apoptosis and tissue damage. A sustained increase in ROS can promote endothelial cell apoptosis and activate inflammatory responses through the activation of MAPKs and NF-κB signaling pathway [19]. *In vivo* experiments have shown that MAPK regulates Ace2 mRNA expression in the aortic vascular smooth muscle cells of rats, indicating that MAPKs may play a role in repairing vascular endothelial injury. [20]. However, the signaling pathway that initiates CRT-induced oxidative stress injury in rats remains unclear.

Resistance exercise is a form of intervention that has been repeatedly validated to reduce the risk of cardiovascular diseases [21]. However, the underlying molecular mechanism by which resistance exercises regulate CRT occurrence has not been elucidated yet. This study investigated the effect of resistance exercise on CRT-induced thrombosis formation. Mechanistically, resistance exercise mitigated oxidative stress and inflammation via regulating miR-92a-3p expression and MAPK/NF-κB pathway.

Materials and Methods

Animals

Fifty male Sprague Dawley rats weighing 190–240 g were purchased from the Experimental Animal Center of Guangxi Medical University (Experimental Animal Breeding License Number SCXK Gui 2014-0002). The rats were exposed to 12/12 light/dark cycles in a strictly controlled environment at 21 °C–23 °C and 50%–60% humidity. As recommended by the Specific Pathogen Free (SPF) barrier environmental conditions, food, bedding, drinking water, and cage devices were thoroughly sterilized. Feeding, disposal of dead experimental animals, and access of the experimenters to the animals were meticulously performed according to SPF laboratory regulations. This study was conducted in accordance with the Guidelines for Ethical Review of Experimental Animals for Animal Welfare in China and was approved by the Animal Protection and Welfare Committee of Guangxi Medical University. This protocol faithfully complied with the Guidelines for Laboratory Animal Care and Use of the National Institutes of Health (NIH Publication No. 85-23). The study was carried out in compliance with the ARRIVE guidelines.

Grouping and Establishment of CRT models

The SD rats were randomly divided into five groups (10 rats in each group) by using a random number generator as follows: control, sham operation (Sham), sham operation + resistance exercise (Sham+RE), CRT, and CRT + resistance exercise (CRT+RE) groups. The rats were intraperitoneally injected with 1.5 mL/kg of 3% sodium pentobarbital solution under general anesthesia. The skin of the right neck was shaved and disinfected with iodopor. All surgical instruments were steam-sterilized, and a disposable sterile orifice plate was used to establish a sterile area around the neck. The catheterization method described by Smith et al. [22] was modified herein to construct CRT models of the SD rats. Surgery was not performed in the rats in the control group. The right external jugular vein of each rat in the Sham and Sham+RE groups was separated after the neck skin was cut open, into which a catheter 3–3.5 cm in length was inserted to destroy the vascular endothelium. The catheter was quickly pulled out, and the wound was immediately sutured closed to stop the bleeding. Each rat in the CRT and CRT+RE groups was incised with blunt dissection of the neck skin and the surrounding connective tissues. An oblique incision was made in the right external jugular vein, and a catheter 3–3.5 cm in length was inserted until it reached the superior

vena cava (Figure 1). The syringe connected to the end of the catheter was pumped. After blood reflux and smooth injection with 0.5 mL of normal saline, the catheter near the heart was ligated and fixed. The tube was sealed with a special plug and placed under the skin, and the subcutaneous tissues and skins were sutured. The rats were kept warm and observed until they recovered from the effects of anesthesia after the operation. Afterward, the rats were fed normally.

Resistance Exercise Intervention in Rats

All rats underwent 1 week of adaptive training before surgery without weight crawling. The rats that completed six times of crawling per day were included in the experiment; otherwise, they were excluded. The rats in the Sham+RE and CRT+RE groups started resistance exercise on the first day after the operation. The rats were trained in climbing a ladder with a gradient of 85° by attaching heavy weights to the tail [23]. The rats were induced to climb from the bottom of the ladder to the top and trained five days a week and rest two days. Two groups were trained every day three times per group for 2 min each time. The rats were allowed to rest at the top of the ladder for 20 s each time. The training was conducted for 8 weeks. The initial weight bearing was 10% of the rats' body weight, and it was gradually increased every succeeding week. In the first, second, third, and fourth weeks, the weight bearing was equivalent to 10%, 20%, 30%, and 40% of the rats' body weight, respectively. The weight bearing was increased to 70% of the rat's body weight from the fifth week to the eighth week and then maintained thereafter [24]. The tails of the rats were stimulated to promote exercise as necessary (Figure 1).

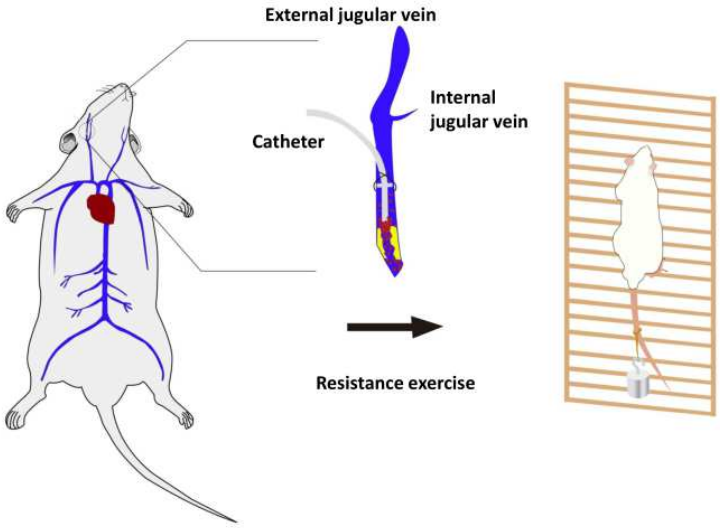


Fig.1 A catheter was inserted into the external jugular vein of the rat. The joint of the catheter was sutured to secure the catheter in proper place. The vascular catheter was fixed to the subcutaneous tissue with sutures, and the second day after the operation, resistance exercise training was started.

Histological Observation

Eight weeks later, rats were euthanized with an overdose of pentobarbital, and the right external jugular veins were removed and fixed overnight with 10% neutral buffer formalin. After soaking and fixation, the catheters in the CRT and CRT+RE groups were slowly removed from the vein before dehydration. Paraffin-embedded tissues were cut into five cross-sections. All tissue sections were stained with hematoxylin and eosin, and thrombosis was inspected by pathologists. Images of the stained sections sealed with neutral gum were digitized using a microscope (BX53, Olympus, Japan). The images were captured using the digital management software cellSens Standard.

ELISA

Serum was isolated from each rat and stored at −80°C for ELISA. ROS ELISA kit (ML0262881, Mlbio, Shanghai, China), MDA ELISA kit (ML022446, Mlbio, Shanghai, China), and HO-1 ELISA kit (ML003108, Mlbio, Shanghai, China) were used to detect the corresponding molecule expression levels in the serum. ELISA experiments were performed according to the manufacturer's instructions.

Quantitative Polymerase Chain Reaction

Total RNA was extracted and purified from 1 cm thick vascular tissues (Ambition, Carlsbad, USA) using a Trizol reagent homogenizer. miRNAs were extracted using miRcute miRNA

extraction and separation kits following the manufacturers' protocols (DP501, Tiangen Biotech). Total RNA was reverse transcribed into cDNA using a miRcute-enhanced miRNA cDNA first-strand synthesis kit (KR211-02, Tiangen Biotech). RT-PCR analysis was performed using a miRcute-enhanced miRNA quantitative fluorescence kit and a SYBR qPCR Mix (Monad, Wuhan, China). The primers used for qPCR are presented in Table I. mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method and normalized to the internal reference gene β-actin or U6.

Table 1. Primer sequences for qPCR

Gene	Forward primer (5'-3')	Reverse prime (5'-3')
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
miR-92a-3p	ATAACGTGAACAGGGCCG	CAGTGCGTGTCGTGGAGT
β-actin	CGTAAAGACCTCTATGCCAACA	TAGGAGCCAGGGCAGTAATC
HO-1	CAGAAGAGGCTAAGACCGCC	GGGGCCAACACTGCATTTAC

Western Blot Analysis

Total protein was extracted from venous tissues of rats in each group were lysed with RIPA lysis buffer. Protein concentration was determined via the BCA method (Solarbio, PC0020). Proteins were separated via SDS-PAGE and transferred onto PVDF membranes. The membranes were incubated at 4˚C overnight with the following primary antibodies in 5% BSA: anti-NF-κb P65 (Phosphoo S536 and AB86299, 1:2000; Abcam), anti-NF-KB P65 (AB16502, 1:2000; Abcam), recombinant anti-IκB alpha (E130) (AB32518, 1:1000; Abcam), P38 MAPK (D13E1) XP® Rabbit mAb (CST 8690, 1:1000), phospho-p38 MAPK (Thr180/Tyr182) (12F8) Rabbit mAb (CST 4631, 1:1000), anti-Ho-1 (K002131P, 1:1000; Solarbio), and anti-β actin (AB8227, 1:500; Abcam) antibodies. The blots were washed thrice with TBST and then incubated with a secondary antibody (goat anti-rabbit: ab6721, 1:4000; Abcam) for 1 h. An ECL reagent was used to render the membrane, and exposure imaging was performed in a gel imager.

Statistical Analysis

Data were analyzed using SPSS 23.0. Data were presented as mean ± standard deviation. Differences among groups were analyzed by one-way ANOVA with Tukey's post hoc test, and rates among groups were compared by Chi-squared test. Pearson correlation analysis was

performed using GraphPad Prism 8.3. *P* values <0.05 were considered statistically significant.

Results

Effects of Resistance Exercise on Rat Body Weight

No significant differences in body weight were observed among the groups from week 0 to week 3 (P > 0.05). By the fourth week, the rats in the Sham+RE group had a significantly lower body weight than the rats in the Sham group (P < 0.05). Moreover, the rats in the CRT+RE group had a significantly lower body weight than those in the CRT group (P < 0.05). From the fifth week to the eighth week, the body weight of the rats in the Sham+RE and CRT+RE groups gradually decreased than that of the rats in the control, Sham, and CRT groups, and the difference was statistically significant (P < 0.01) (Table 2). The body weight of the rats increased as time progressed, but their weight was reduced by the anti-resistance exercise training, thereby confirming the effectiveness of the exercise training program (Figure 2).

Table2.Weight of rats in each group from week five to week eight

Time (weeks)	Control(n=10,g)	Sham(n=10,g)	Sham+RE(n=10,g)	CRT(n=10,g)	CRT+RE(n=10,g)
The fifth	346.90±36.27**##	362.50±32.51*###	308.80±27.45	356.40±39.52#	313.10±12.75
The sixth	383.20±38.11**##	396.50±35.57*###	327.10±26.18	388.70±41.70#	335.80±7.74
The seventh	420.20±32.61**##	426.30±35.51*###	352.60±23.62	428.40±43.01#	357.30±11.03
The eighth	471.50±33.90**##	459.8±36.32*###	369.70±26.74	461.20±36.84#	380.00±10.81

Values are expressed as mean ± standard deviation(n = 10 per group) Control groups； sham groups；Sham+RE groups;CRT groups; CRT+ RE，One factor analysis of variance, ** P<0.01,VS. Sham+RE,## P<0.01,VS. CRT+ R,### P<0.01 CRT+ RE,* P<0.01,VS. Sham+RE,#P<0.01.VS.CRT+RE

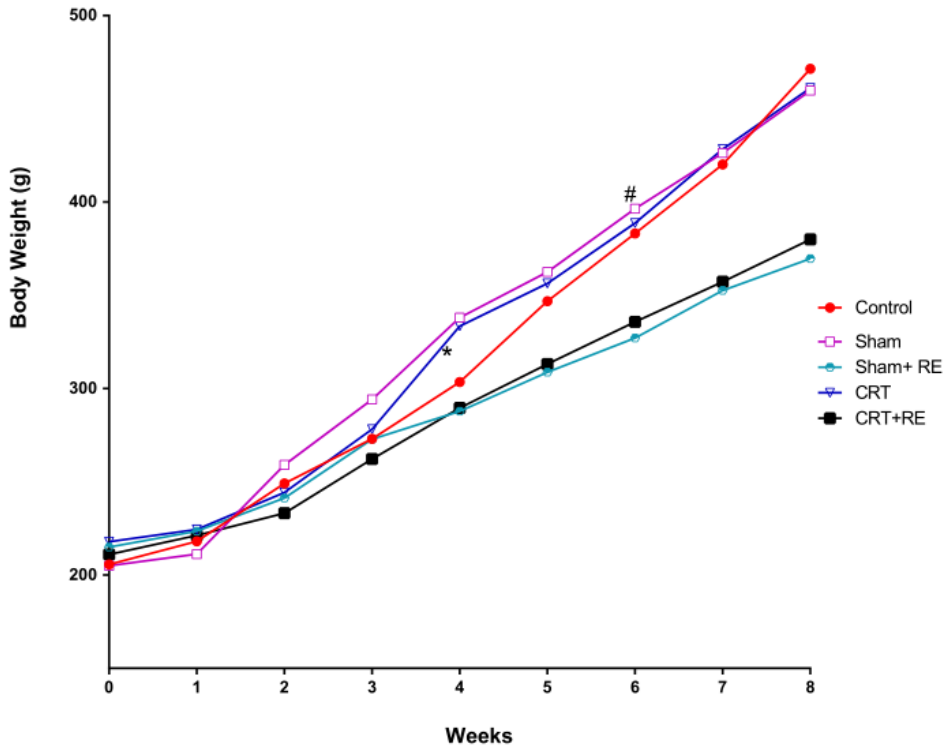


Fig.2 Weight of rats in each group at 8 weeks. Values are expressed as mean ± standard deviation (n = 10 per group)， Control groups；sham operation groups；Sham+Resistance exercise groups; CRT groups; CRT+ Resistance exercise，one factor analysis of variance, Four to eight weeks # P<0.01,VS. sham+RE groups； * P<0.01,VS.CRT+RE

CRT Formation in Rats

The success rate of indwelling catheter was 100%, and the catheter did not fall off during indwelling. All rats survived. No thrombosis was observed in the control, Sham, and Sham+RE groups. In the CRT group, the success rate was 90% (9/10), whereas only a small amount of thrombosis with a formation rate of 30% (3/10) was observed in the CRT+RE group. Compared with that in the CRT group, the number of thrombosis cases in the CRT+RE group decreased, and the difference was statistically significant ($P < 0.05$). Histological features were also explored in this study. In the control, Sham, and Sham+RE groups, smooth and intact venous endothelial cells were observed under an optical microscope, and no thrombosis was found in the vascular lumen. Small amounts of powder were observed in the lumen of the rats in the Sham+RE group, but no thrombosis was observed. In the CRT group, inflammatory cells infiltrated around the vessel wall and formed thrombus, and no endothelial cells were present at the adhesion site. In the CRT+RE group, a small number of red blood cells, white blood cells, and platelet beams gathered in the lumen, and the severity

of thrombus was less than that in the CRT group. The CRT in the CRT group was observed at the edge of the catheter. Trabecular platelets, red blood cells, and white blood cells were found in the thrombus (Figure 3).

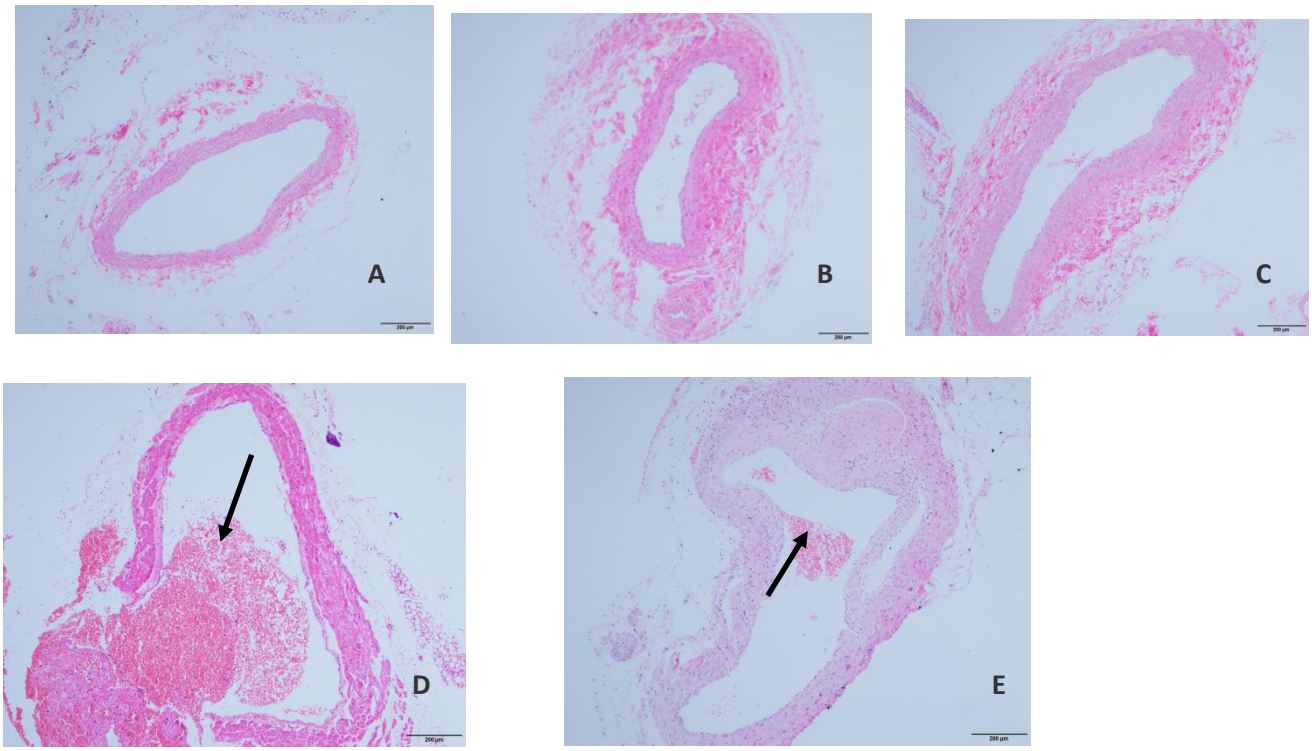
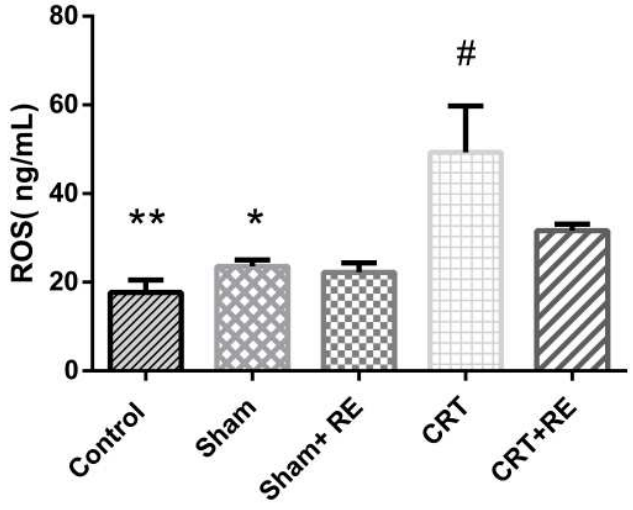


Fig.3 A: Control groups;B: sham operation groups;C: Sham+RE groups D: CRT groups; E: CRT+ RE. The black arrow represents the CRT

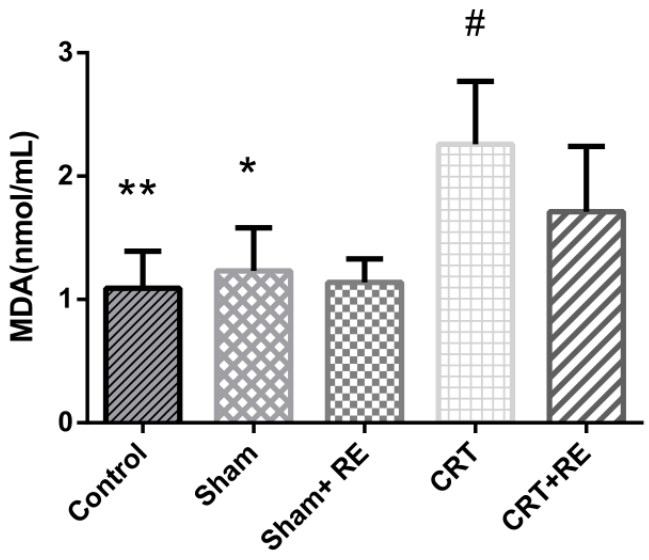
Levels of Oxidative Stress in Rats

Compared with those in the control group, the oxidative stress levels of ROS and MDA in the CRT group significantly increased (P<0.01), whereas that of HO-1 significantly decreased (*P*<0.01).Compared with those in the CRT group, the oxidative stress levels of ROS and MDA in the CRT+ RE group significantly decreased, whereas that of HO-1 significantly increased (Figure 4). Compared with those in the Sham group, the oxidative stress level of Sham+RE group was not statistically significant (*P*>0.05).

A


80
60
40
20
0
ROS(ng/mL)
**
*
#
Control
Sham
Sham+ RE
CRT
CRT+RE

B


3
2
1
0
MDA(nmol/mL)
**
*
#
Control
Sham
Sham+ RE
CRT
CRT+RE

C

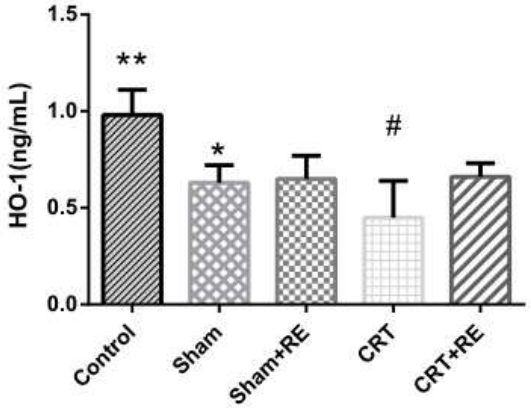


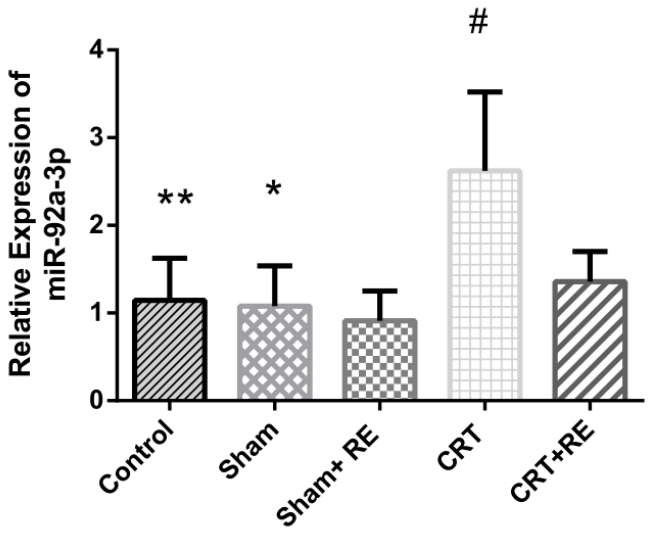
Fig.4 The expression levels of ROS(A), MDA (B) and HO-1(C) in serum of rats.

** p<0.01,VS .CRT ,* P＞**0.05**,Vs. Sham+RE,# P <0.01,VS. CRT+ RE.

Expression Levels of miR -92a-3p and HO-1 mRNA in Rat Venous Tissues

The expressions of miR-92a-3p and HO-1 mRNA in rat tissues were detected via RT-PCR. Their expressions in the CRT group were significantly different from those in the control group (P < 0.01). Moreover, differences in miR-92a-3p and HO-1 mRNA expressions between the CRT CRT+RE groups were statistically significant (P < 0.01) (Figure 5). However, no statistically significant difference was observed between the Sham and Sham+RE groups (P > 0.05).

A



B

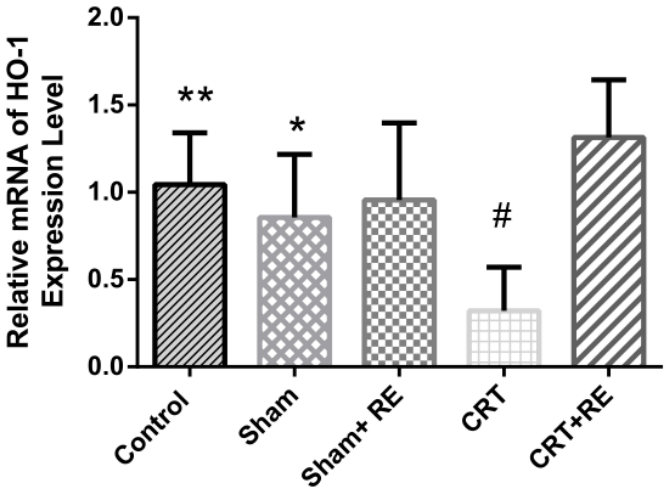


Fig.5 Expression of miR-92a-3p (A) and HO-1 (B) mRNA in rat tissues

**p < 0.01,VS. CRT groups, *P＞0.05, VS. Sham+RE,# p < 0.01,VS CRT+ RE groups.

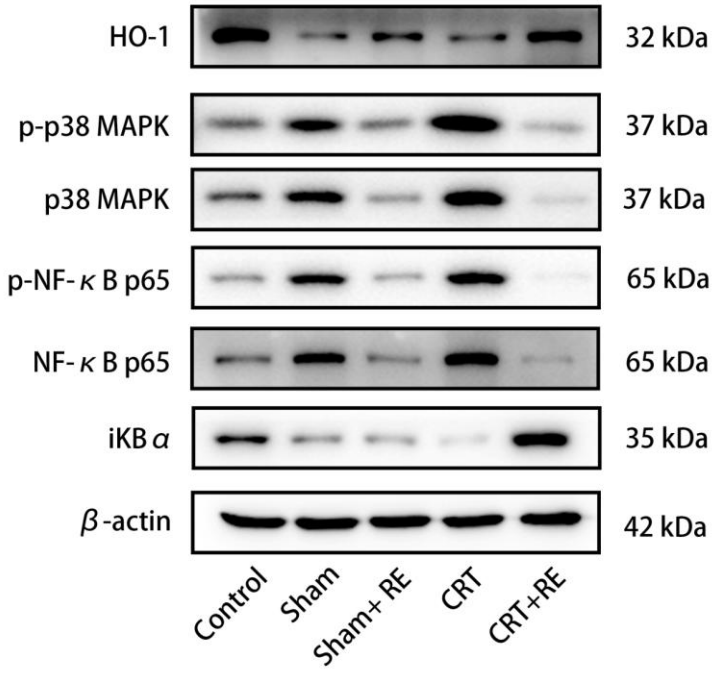
Correlation Analysis between miR-92a-3p and HO-1

MiR-92a-3p was negatively correlated with HO-1 in rat venous tissues (r = −0.4197, P < 0.01).

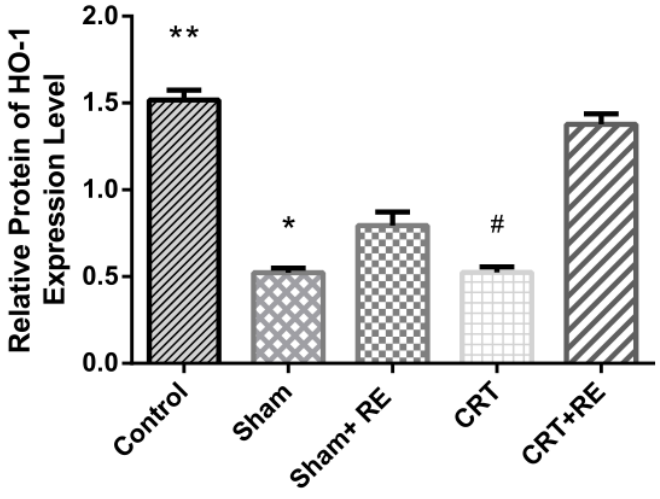
Expressions of HO-1 and MAPK/NF-κB Pathway Proteins

According to the results of Western blot analysis, compared with that in the control group, the expression level of HO-1 in the CRT group decreased, the phosphorylation level of P38 MAPK and phosphorylation P38 significantly increased, the phosphorylation levels of NF-κB p65 and NF-κB P65 significantly increased, and the IκBa expression level significantly decreased. Moreover, differences in the phosphorylation levels of HO-1, P38 MAPK, phosphorylation p38, and NF-κB P65 between the Sham and Sham+RE groups were statistically significant, whereas differences in the levels of NF-κB P65 and IκBa were similar but not statistically significant. Furthermore, compared with that in the CRT group, the expression level of HO-1 in the CRT+RE group increased, the phosphorylation levels of p38 MAPK and phosphorylated P38 significantly decreased, the phosphorylation levels of NF-κB p65 and NF-κB P65 significantly decreased, and the expression level of IκBa significantly increased in the CRT+RE group (Figure 6).

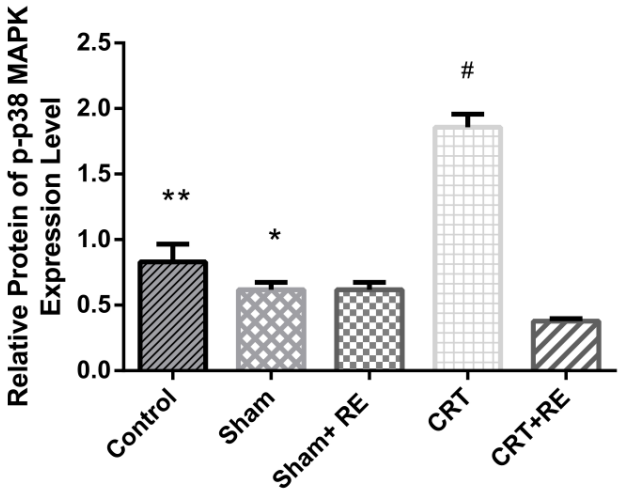
A


HO-1
32 kDa
p-p38 MAPK
37 kDa
p38 MAPK
37 kDa
p-NF-κ B p65
65 kDa
NF-κ B p65
65 kDa
iKB α
35 kDa
β-actin
42 kDa
Control
Sham
Sham+ RE
CRT
CRT+RE

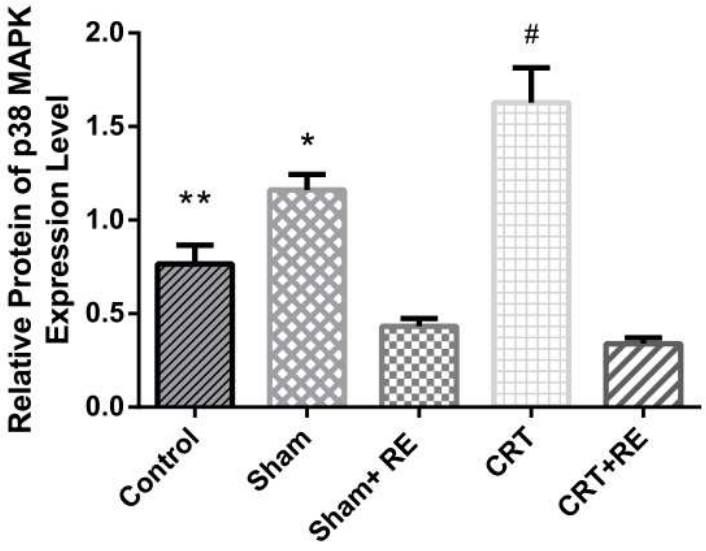
B


Relative Protein of HO-1 Expression Level
2.0
1.5
1.0
0.5
0.0
**
*
#
Control
Sham
Sham+ RE
CRT
CRT+RE

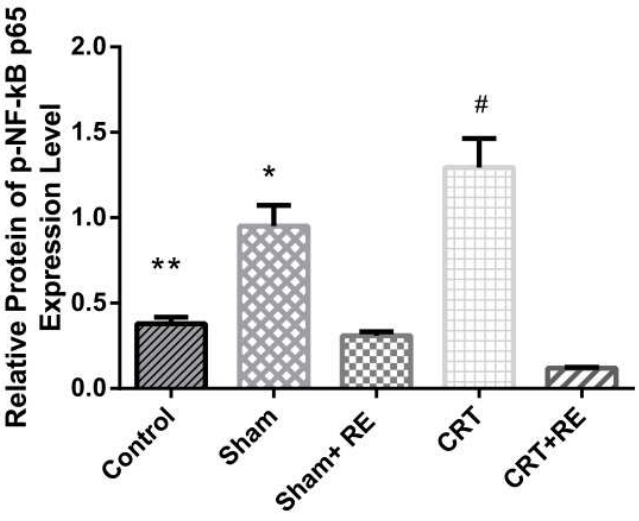
C


Relative Protein of p-p38 MAPK Expression Level
2.5
2.0
1.5
1.0
0.5
0.0
**
*
#
Control
Sham
Sham+ RE
CRT
CRT+RE

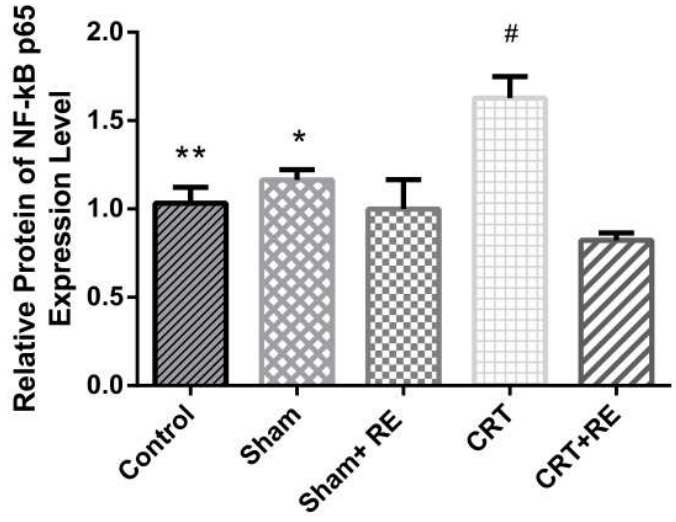
D


Relative Protein of p38 MAPK Expression Level
2.0
1.5
1.0
0.5
0.0
**
*
#
Control
Sham
Sham+ RE
CRT
CRT+RE

E


Relative Protein of p-NF-kB p65 Expression Level
2.0
1.5
1.0
0.5
0.0
**
*
#
Control
Sham
Sham+ RE
CRT
CRT+RE

F



G

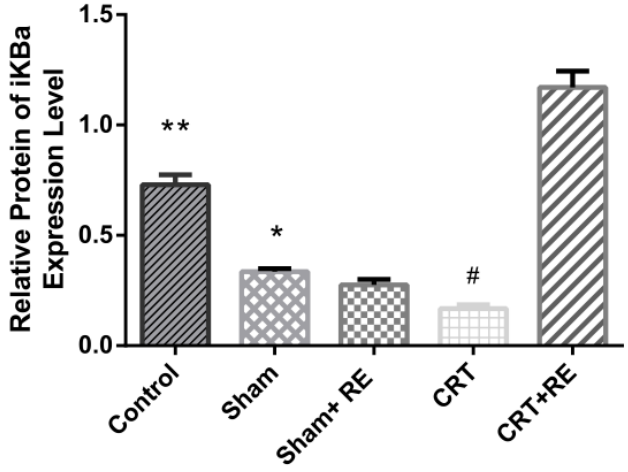


Fig.6 A Western blot image of HO-1、p-p38MAPK、p38 MAPK, P-NF-κB p65、NF-κB p65、IkBa .

B-G shows the analysis of protein expression of HO-1、p-p38MAPK、p38 MAPK, P-NF-κB p65、NF-κB p65、Iκba， **p < 0.01,VS. CRT groups；#p < 0.01,VS. CRT+ RE groups.B-E HO-1、p38 MAPK、p-p38 MAPK and p-NF-kB p65,*p< 0.01,VS.Sham+RE groups.

Discussion

The incidence of thrombosis is the highest among all diseases, and its incidence rate is increasing. Via resistance exercise intervention, this study investigated the mechanism by which oxidative stress and miR-92a-3p prevent catheter-related thrombosis in rats. Results showed that blood flow direction and velocity changed after catheter implantation, resulting in thrombosis formation, increasing ROS production and MDA activity, decreasing HO-1 level, and increasing miR-92a-3p expression. CRT also increased the release of proinflammatory cytokines, activation of P38MAPK/NF-κB P65 pathway, and inhibited Iκba expression.

Oxidative stress refers to the excess ROS produced by the body when it is adversely stimulated, resulting in imbalance between oxidative and antioxidant systems, which in turn leads to the accumulation of free radicals in cells and cell damage [25]. ROS content is an important marker of oxidative stress injury and also one of the important factors that lead to thrombosis occurrence and development [26]. In the cardiovascular system, ROS play a role in controlling inflammation, proliferation, apoptosis, endothelial function, and angiogenesis [27]. Aizawa et al. [26] demonstrated that an increase in ROS production in endothelial cells can promote thrombosis formation, leading to endothelial cell dysfunction. High ROS contents can peroxide polyunsaturated fatty acids on the cell membrane and produce MDA, thus further causing oxidative stress-induced tissue damage, increasing membrane permeability, and rupturing the double-layered structure of the membrane.

Most miRNAs are effective therapeutic targets for cancer, but their roles in the cardiovascular system are not entirely clear [28]. As a member of the miR-17-92 family (i.e., miR-17, -18a, -19a/B, -20A, and miR -92a), miR-92a-3p is highly expressed in vascular endothelial cells and participates in the regulation of vascular endothelial functions [29-31]. A sharp decrease in blood flow shear stress after catheter placement in the external jugular vein led to upregulation of miR-92a-3p expression, vascular endothelial cell proliferation, apoptosis and thrombogenic molecular secretion, and CRT formation [32]. As a member of the HO protein family, HO-1 is a common mammalian induction enzyme that degrades oxyhemoglobin and produces antioxidants to protect cells from oxidative stress damage [33]. Pro-apoptotic pathways (e.g., MAPKs) and inflammatory response pathways (e.g., NF-κB) are important in regulating apoptosis and tissue damage. MAPKs and NF-κB are sensitive ROS signaling pathways. A sustained increase in ROS production can promote endothelial cell apoptosis and activate inflammatory responses [19]. NF-κB is maintained in its inactive form in the cytoplasm by binding to an inhibitory protein of the IκB family. In turn, this protein is phosphorylated and degraded under inflammatory stimuli, allowing the transfer of NF-κB p65 subunit phosphorylation and NF-κB dimer to the nucleus [34]. Punctures or long-term catheter placement in the vascular wall can cause oxidative stress injury in vascular endothelial cells. Moreover, they affect the biological functions of endothelial cells, suggesting that CRT and OS injury has a close link, a supposition consistent with the CRT

model in the present study. Numerous studies confirmed that upper limb exercises can effectively promote blood circulation and reduce CRT occurrence[35]. In the current study, the resistance exercise intervention conducted for 8 weeks reduced ROS production and MDA activity, increased HO-1 level, and downregulated miR-92a-3p expression. Furthermore, the intervention reduced the P38MAPK/NF-κB P65 pathway and their phosphorylation levels and activated IκBa expression.

Thrombosis is a disease that involves numerous factors and systems. Clinical treatment of CRT remains a difficult task for clinicians. Anticoagulants [36], catheter surface coating [37], compression therapy [38], and grip strength training are commonly used in clinical settings [4]. Resistance exercise, also known as resistance training or strength training, usually refers to the process by which the body overcomes resistance to achieve muscle growth and strength increase[8]. Clinical grip strength training is actually a kind of resistance exercise training. Resistance exercises upregulate the antioxidant defense system, decrease the concentration of cellular ROS, and confer protection against oxidative stress-related diseases. The present study also proved that antiresistance exercises changed blood flow shear stress, inhibited endothelial cells to repair oxidative stress, and reduced ROS production, thereby improving the function of vascular endothelium and, to a certain extent, played a role in preventing venous thrombosis [39, 40]. These results were consistent with the findings of Quinteiro [41].

By monitoring changes in miRNA expression, researchers found that miRNA circulation also changes as the intensity of resistance exercises increases [42, 43]. The current study showed that the resistance exercise intervention downregulated miR-92a-3p expression, affected endothelial cell expression, repaired oxidative stress damage, and inhibited CRT formation. Enhancing HO-1 activity by inhibiting the action of miR-92a can reduce oxidative stress damage and improve endothelial functions [32]. Intravenous upregulation of miR-92a induces oxidative stress in endothelial cells, leading to endothelial inflammation and dysfunction [27]. We speculated that miR-92a-3p and HO-1 have a certain correlation with CRT. Results showed that miR-92a-3p was positively correlated with HO-1. However, the target of HO-1 regulation of thrombosis was not comprehensively explored. Therefore, the supposition that resistance exercise may mediate the targeting of HMOX1 by miR-92a-3p to

regulate oxidative stress and prevent CRT occurrence warrants further experimental verification.

Shear stress is also a strong inducer of HMOX1 that can inhibit leukocyte adhesion and platelet aggregation. HO-1 can inhibit the formation of venous thrombosis by alleviating oxidative stress mechanisms [44]. However, the role of HO-1 in reducing tissue damage, especially in venous thrombosis, through its antioxidant activity is poorly understood. Recent studies explored potential therapeutic tools for manipulating apoptosis, inflammation, and oxidative stress to improve the outcome of vascular diseases. Studies of the HO-1 promoter region revealed that the combination of the presence of transcriptional response elements, including activator protein I, activator protein II, NF-κB, interleukin-6 response elements, with antioxidant response elements, induced the inhibition of the proliferation of vascular smooth muscle cells [45]. Aside from the fact that HO-1 expression is induced by oxidative stress, HO-1 also plays an important cellular protective role in various inflammatory diseases. Previous studies explored the mechanism of oxidative stress in rat brain as a function of age. Western blot and immunohistochemistry analyses revealed that the expression level of the HO-1 protein in the heart antioxidant enzyme slightly decreased [46], whereas that in the kidney slightly increased. However, HO-1 expression in the kidney substantially decreased by the fifth week [47]. Therefore, HO-1 expression is slightly different in different tissues and at different time points. Nevertheless, HO-1 was undeniably structurally upregulated among the top climbers, and the high HO-1 expression level was maintained months after reaching the peak [48]. Eight weeks of climbing resistance training induced an increase in HO-1 expression, which in turn induced an antioxidant reaction in the blood vessels, thereby initiating the corresponding protective mechanism.

Limitations and Prospects

This study provides a theoretical basis for CRT prevention via resistance exercise intervention. This process is important in future translational research, especially for patients in ICUs or with cancer undergoing chemotherapy. Nevertheless, this study has several limitations. First, thrombosis was only assessed by pathology. If catheterization and CRT monitoring can be combined with ultrasound technology, thrombosis formation and the relationship between blood flow changes and CRT can be determined in various forms. Second, platelet activation

was ruled out; however, the clotting system may be involved in CRT formation. We monitored thrombus formation at different time periods spanning 14 days and at the end of 2 months [49]. We will continue examining changes in thrombus formation at different time periods for 1 month under resistance exercise intervention. Moreover, we will constantly track the effects of changes in resistance exercise training at different time points on HO-1 and promoter regions, especially on the protection and improvement of blood vessels.

Conclusion

Vascular catheterization resulted in endothelial cell oxidative stress damage and miR-92a-3p-mediated inflammation, ultimately leading to thrombosis. Resistance exercise intervention accelerated blood flow speed, repaired oxidative stress damage, reduced ROS production, and reduced CRT incidence, thereby improving cardiovascular functions. Resistance exercises are evidently important in CRT prevention and treatment. Resistance exercise may mediate the regulation of oxidative stress through the targeting of HMOX1 by miR-92a-3p to prevent CRT occurrence. However, this supposition warrants further research. Resistance exercise intervention provides a strong theoretical basis and research direction for CRT prevention and treatment.

Abbreviations

CVC:Central venous catheter; CRT: Catheter-related thrombosis;ROS: Reactive oxygen species; MDA: Malondialdehyde; HO-1: Heme oxygenase-1; miR-92a-3p: MicroRNA-92a-3p; P38 MAPK: p38-mitogen-activated protein kinase; NF-κB: Nuclear factor kappa-B; ELISA: Enzyme-linked immunosorbent assay; qPCR: Quantitative polymerase chain reaction

Declaration

Acknowledgment

I would like to thank Danming Wei and Hua Nong for their guidance and supervision of this research.

Authors' contributions

CW and YY conceived the study and designed the experiment. CW,QJ, XG, YW,JW, XH and made substantial contributions to the experiment and acquisition of data. CW analyzed data and wrote the manuscript. HZ and YY design and revised the manuscript critically for important intellectual content. YY gave the final approval of the version to be published. All

authors read andapproved the final version of the manuscript.

Funding

This work was financially supported by the National Science Foundation of China (Grant No. 81860032) and Guangxi Natural Science Foundation project （ Grant No.2018GXNSFAA050081）.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Ethics approval and consent to participate conformed to the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health(NIH Publication No. 85–23). And the experiment protocol was approved by the Ethics Committee of Animal Care and Welfare Committee at Guangxi Medical University, Nanning, China.

Consent for publication

Not applicable

Competing interests

The authors declare no conflicts of interest.

Reference

1. Gavin NC, Webster J, Chan RJ, Rickard CM. Frequency of dressing changes for central venous access devices on catheter-related infections. The Cochrane database of systematic reviews. 2016;2:CD009213.

2. Wall C, Moore J, Thachil J. Catheter-related thrombosis: A practical approach. J Intensive Care Soc. 2016;17(2):160-7.

3. Grant JD, Stevens SM, Woller SC, Lee EW, Kee ST, Liu DM, et al. Diagnosis and management of upper extremity deep-vein thrombosis in adults. Thrombosis and haemostasis. 2012;108(6):1097-108.

4. Liu K, Zhou Y, Xie W, Gu Z, Jin Y, Ye X, et al. Handgrip exercise reduces peripherally-inserted central catheter-related venous thrombosis in patients with solid cancers: A randomized controlled trial. International journal of nursing studies. 2018;86:99-106.

5. Hegerova L, Bachan A, Cao Q, Vu HX, Rogosheske J, Reding MT, et al. Catheter-Related Thrombosis in Patients with Lymphoma or Myeloma Undergoing Autologous Stem Cell Transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2018;24(12):e20-e5.

6. Kamphuisen PW, Lee AY. Catheter-related thrombosis: lifeline or a pain in the neck? Hematology American Society of Hematology Education Program. 2012;2012:638-44.

7. Geerts W. Central venous catheter-related thrombosis. Hematology American Society of Hematology Education Program. 2014;2014(1):306-11.
8. Gorski LA. The 2016 Infusion Therapy Standards of Practice. Home Healthc Now. 2017;35(1):10-8.
9. Colwell CW, Jr., Froimson MI, Mont MA, Ritter MA, Trousdale RT, Buehler KC, et al. Thrombosis prevention after total hip arthroplasty: a prospective, randomized trial comparing a mobile compression device with low-molecular-weight heparin. J Bone Joint Surg Am. 2010;92(3):527-35.
10. Metsios GS, Moe RH, van der Esch M, van Zanten J, Fenton SAM, Koutedakis Y, et al. The effects of exercise on cardiovascular disease risk factors and cardiovascular physiology in rheumatoid arthritis. Rheumatology international. 2020;40(3):347-57.
11. Kietzmann T, Petry A, Shvetsova A, Gerhold JM, Gorlach A. The epigenetic landscape related to reactive oxygen species formation in the cardiovascular system. Br J Pharmacol. 2017;174(12):1533-54.
12. C D, A B, A F, A S, Y R, C U, et al. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. 2010;115(23):4944-50.
13. Boon RA, Hergenreider E, Dimmeler S. Atheroprotective mechanisms of shear stress-regulated microRNAs. Thrombosis and haemostasis. 2012;108(4):616-20.
14. Liu Y, Li Q, Hosen MR, Zietzer A, Flender A, Levermann P, et al. Atherosclerotic Conditions Promote the Packaging of Functional MicroRNA-92a-3p Into Endothelial Microvesicles. Circulation research. 2019;124(4):575-87.
15. Jin QQ, Sun JH, Du QX, Lu XJ, Zhu XY, Fan HL, et al. Integrating microRNA and messenger RNA expression profiles in a rat model of deep vein thrombosis. International journal of molecular medicine. 2017;40(4):1019-28.
16. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol. 2014;24(10):R453-62.
17. Papac-Milicevic N, Busch CJ, Binder CJ. Malondialdehyde Epitopes as Targets of Immunity and the Implications for Atherosclerosis. Advances in immunology. 2016;131:1-59.
18. Choi YK. Role of Carbon Monoxide in Neurovascular Repair Processing. Biomolecules & therapeutics. 2018;26(2):93-100.
19. Fan H, Wu PF, Zhang L, Hu ZL, Wang W, Guan XL, et al. Methionine sulfoxide reductase A negatively controls microglia-mediated neuroinflammation via inhibiting ROS/MAPKs/NF-kappaB signaling pathways through a catalytic antioxidant function. Antioxidants & redox signaling. 2015;22(10):832-47.
20. Gallagher PE, Ferrario CM, Tallant EA. MAP kinase/phosphatase pathway mediates the regulation of ACE2 by angiotensin peptides. American journal of physiology Cell physiology. 2008;295(5):C1169-74.
21. Fihn SD, Gardin JM, Abrams J, Berra K, Blankenship JC, Dallas AP, et al. 2012 ACCF/AHA/ACP/AATS/PCNA/SCAI/STS Guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, and the American College of Physicians, American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. Journal of the American College of Cardiology. 2012;60(24):e44-e164.
22. Smith MA, Schmidt KT, Iordanou JC, Mustroph ML. Aerobic exercise decreases the positive-reinforcing effects of cocaine. Drug Alcohol Depend. 2008;98(1-2):129-35.

23. Kim JY, Choi MJ, So B, Kim HJ, Seong JK, Song W. The Preventive Effects of 8 Weeks of Resistance Training on Glucose Tolerance and Muscle Fiber Type Composition in Zucker Rats. Diabetes & metabolism journal. 2015;39(5):424-33.
24. Koo JH, Kang EB, Cho JY. Resistance Exercise Improves Mitochondrial Quality Control in a Rat Model of Sporadic Inclusion Body Myositis. Gerontology. 2019;65(3):240-52.
25. Szabo C, Ischiropoulos H, Radi R. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov. 2007;6(8):662-80.
26. Aizawa K, Takahari Y, Higashijima N, Serizawa K, Yogo K, Ishizuka N, et al. Nicorandil prevents sirolimus-induced production of reactive oxygen species, endothelial dysfunction, and thrombus formation. Journal of pharmacological sciences. 2015;127(3):284-91.
27. Chen Z, Wen L, Martin M, Hsu CY, Fang L, Lin FM, et al. Oxidative stress activates endothelial innate immunity via sterol regulatory element binding protein 2 (SREBP2) transactivation of microRNA-92a. Circulation. 2015;131(9):805-14.
28. Gurha P. MicroRNAs in cardiovascular disease. Current opinion in cardiology. 2016;31(3):249-54.
29. Doebele C, Bonauer A, Fischer A, Scholz A, Reiss Y, Urbich C, et al. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. Blood. 2010;115(23):4944-50.
30. Chamorro-Jorganes A, Lee MY, Araldi E, Landskroner-Eiger S, Fernández-Fuertes M, Sahraei M, et al. VEGF-Induced Expression of miR-17-92 Cluster in Endothelial Cells Is Mediated by ERK/ELK1 Activation and Regulates Angiogenesis. Circulation research. 2016;118(1):38-47.
31. Mogilyansky E, Rigoutsos I. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. Cell death and differentiation. 2013;20(12):1603-14.
32. Gou L, Zhao L, Song W, Wang L, Liu J, Zhang H, et al. Inhibition of miR-92a Suppresses Oxidative Stress and Improves Endothelial Function by Upregulating Heme Oxygenase-1 in db/db Mice. Antioxidants & redox signaling. 2018;28(5):358-70.
33. Grochot-Przeczek A, Dulak J, Jozkowicz A. Haem oxygenase-1: non-canonical roles in physiology and pathology. Clin Sci (Lond). 2012;122(3):93-103.
34. Kim JI, Choi SH, Jung KJ, Lee E, Kim HY, Park KM. Protective role of methionine sulfoxide reductase A against ischemia/reperfusion injury in mouse kidney and its involvement in the regulation of trans-sulfuration pathway. Antioxidants & redox signaling. 2013;18(17):2241-50.
35. Cunha PM, Ribeiro AS, Tomeleri CM, Schoenfeld BJ, Silva AM, Souza MF, et al. The effects of resistance training volume on osteosarcopenic obesity in older women. J Sports Sci. 2018;36(14):1564-71.
36. Diamond CE, Hennessey C, Meldau J, Guelcher CJ, Guerrera MF, Conklin LS, et al. Catheter-Related Venous Thrombosis in Hospitalized Pediatric Patients with Inflammatory Bowel Disease: Incidence, Characteristics, and Role of Anticoagulant Thromboprophylaxis with Enoxaparin. The Journal of pediatrics. 2018;198:53-9.
37. De Luca T, Szilágyi KL, Hargreaves KA, Collins KS, Benson EA. Improving the Patency of Jugular Vein Catheters in Sprague-Dawley Rats by Using an Antiseptic Nitrocellulose Coating. Journal of the American Association for Laboratory Animal Science : JAALAS. 2018;57(5):520-8.
38. Partsch H. Compression therapy for deep vein thrombosis. VASA Zeitschrift fur Gefasskrankheiten. 2014;43(5):305-7.
39. Chatzizisis YS, Baker AB, Sukhova GK, Koskinas KC, Papafaklis MI, Beigel R, et al. Augmented

expression and activity of extracellular matrix-degrading enzymes in regions of low endothelial shear stress colocalize with coronary atheromata with thin fibrous caps in pigs. Circulation. 2011;123(6):621-30.

40. Theodoris CV, Li M, White MP, Liu L, He D, Pollard KS, et al. Human disease modeling reveals integrated transcriptional and epigenetic mechanisms of NOTCH1 haploinsufficiency. Cell. 2015;160(6):1072-86.

41. Quinteiro H, Buzin M, Conti FF, Dias Dda S, Figueroa D, Llesuy S, et al. Aerobic exercise training promotes additional cardiac benefits better than resistance exercise training in postmenopausal rats with diabetes. Menopause. 2015;22(5):534-41.

42. Polakovicova M, Musil P, Laczo E, Hamar D, Kyselovic J. Circulating MicroRNAs as Potential Biomarkers of Exercise Response. International journal of molecular sciences. 2016;17(10).

43. Nielsen S, Akerstrom T, Rinnov A, Yfanti C, Scheele C, Pedersen BK, et al. The miRNA plasma signature in response to acute aerobic exercise and endurance training. PloS one. 2014;9(2):e87308.

44. Fei D, Meng X, Zhao M, Kang K, Tan G, Pan S, et al. Enhanced induction of heme oxygenase-1 suppresses thrombus formation and affects the protein C system in sepsis. Translational research : the journal of laboratory and clinical medicine. 2012;159(2):99-109.

45. Hoekstra KA, Godin DV, Cheng KM. Protective role of heme oxygenase in the blood vessel wall during atherogenesis. Biochemistry and cell biology = Biochimie et biologie cellulaire. 2004;82(3):351-9.

46. Qian X, Asad SB, Li J, Wang J, Wei D, Zhao Y, et al. Link between cardiac function and the antioxidative defense mechanism in aged rats. Biochemical and biophysical research communications. 2019;513(4):1100-5.

47. Miyazono M, Garat C, Morris KG, Jr., Carter EP. Decreased renal heme oxygenase-1 expression contributes to decreased renal function during cirrhosis. American journal of physiology Renal physiology. 2002;283(5):F1123-31.

48. Miura G, Kato K, Shimizu T, Shiga D, Shirasawa T. Heme oxygenase-1 (HO-1) is constitutively up-regulated in top alpinists. Biochemical and biophysical research communications. 2012;417(1):104-8.

49. Gan X, Zhao H, Wei Y, Jiang Q, Wen C, Ying Y. Role of miR-92a-3p, oxidative stress, and p38MAPK/NF-κB pathway in rats with central venous catheter related thrombosis. BMC cardiovascular disorders. 2020;20(1):150.

Figures

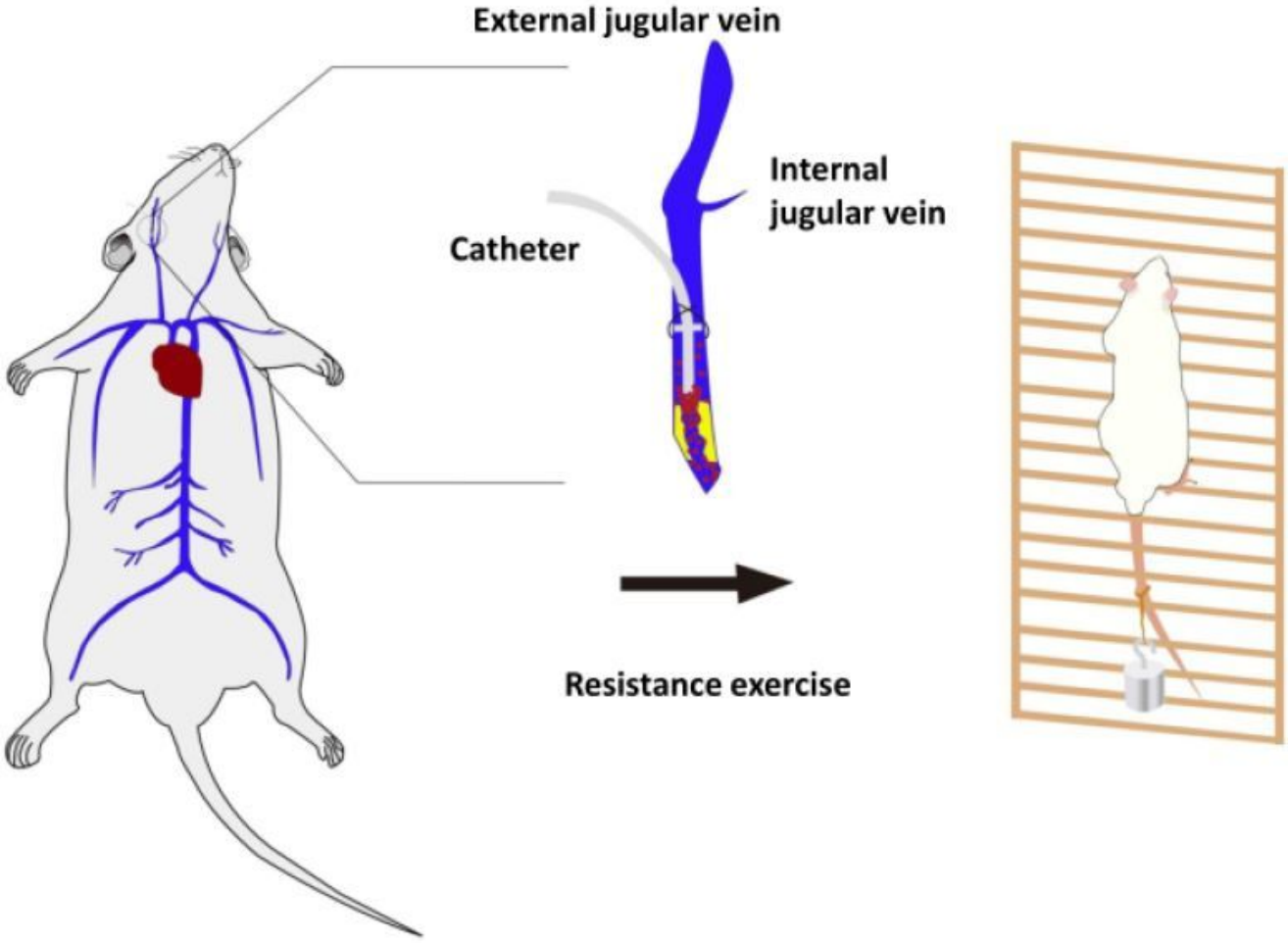


Figure 1

A catheter was inserted into the external jugular vein of the rat. The joint of the catheter was sutured to secure the catheter in proper place. The vascular catheter was fixed to the subcutaneous tissue with sutures, and the second day after the operation, resistance exercise training was started.

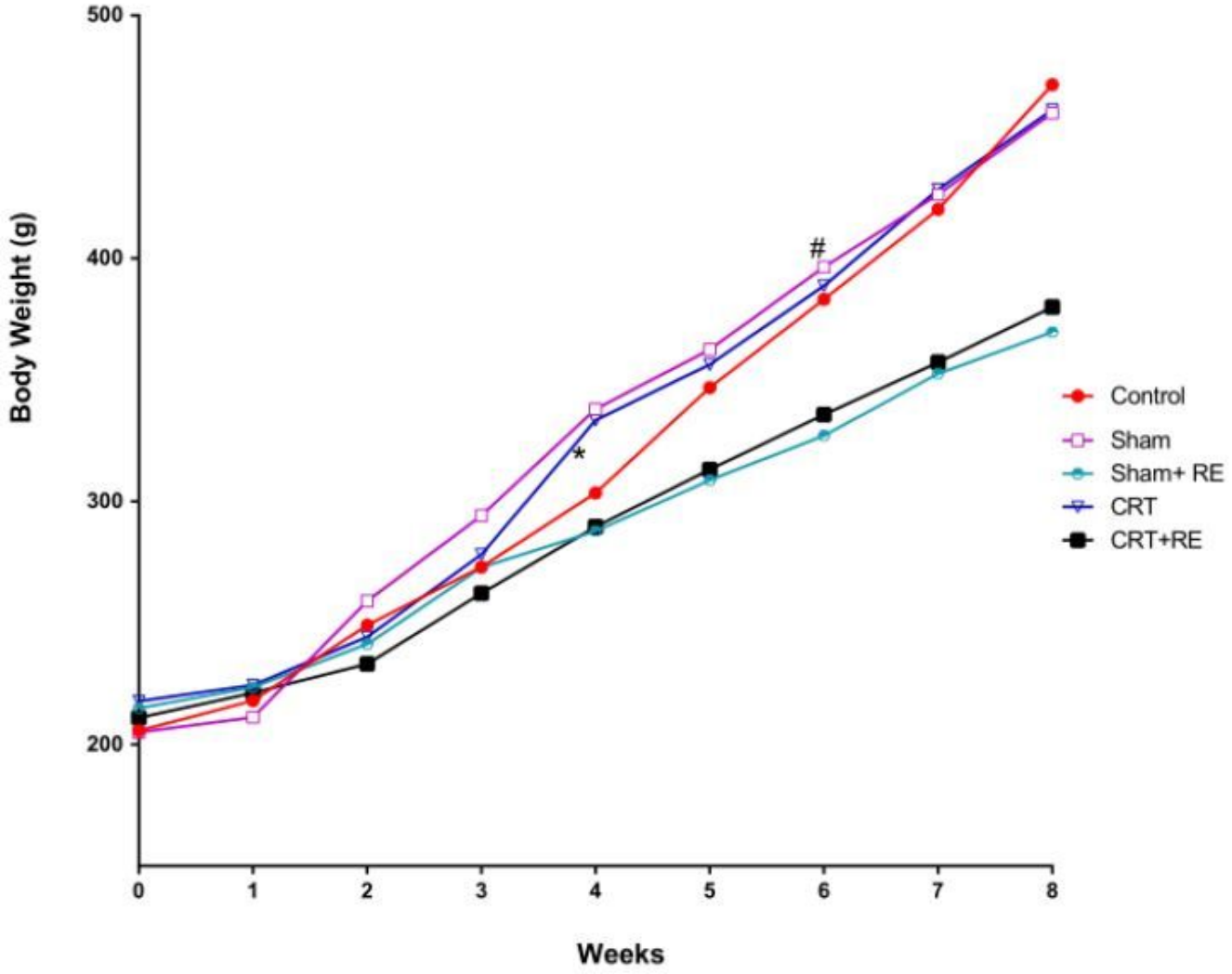


Figure 2

Weight of rats in each group at 8 weeks. Values are expressed as mean ± standard deviation (n = 10 per group) Control groups sham operation groups Sham+Resistance exercise groups; CRT groups; CRT+ Resistance exercise one factor analysis of variance, Four to eight weeks # P<0.01,VS. sham+RE groups * P<0.01,VS.CRT+RE

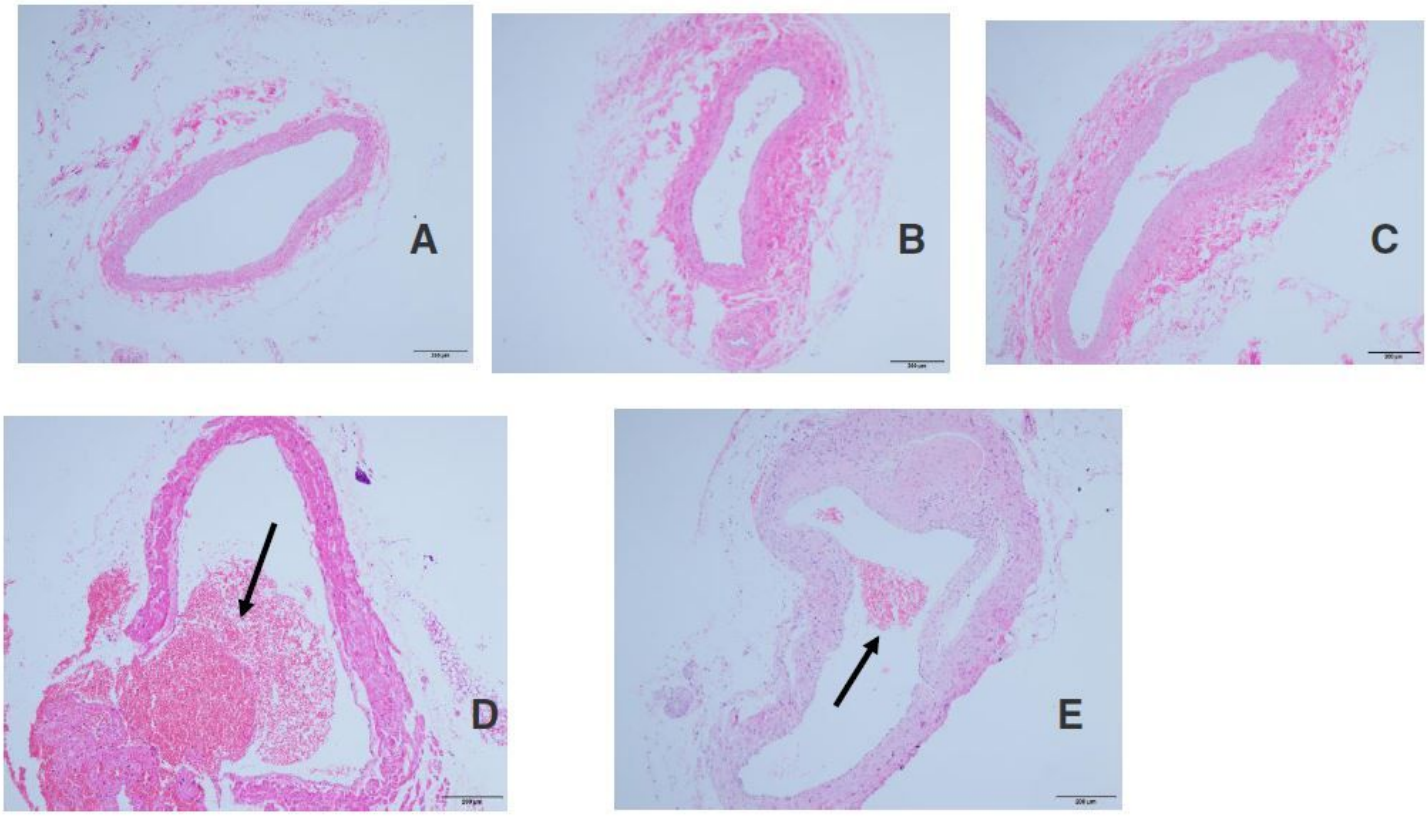


Figure 3

CRT Formation in Rats .A: Control groups;B: sham operation groups;C: Sham+RE groups D: CRT groups; E: CRT+ RE. The black arrow represents the CRT

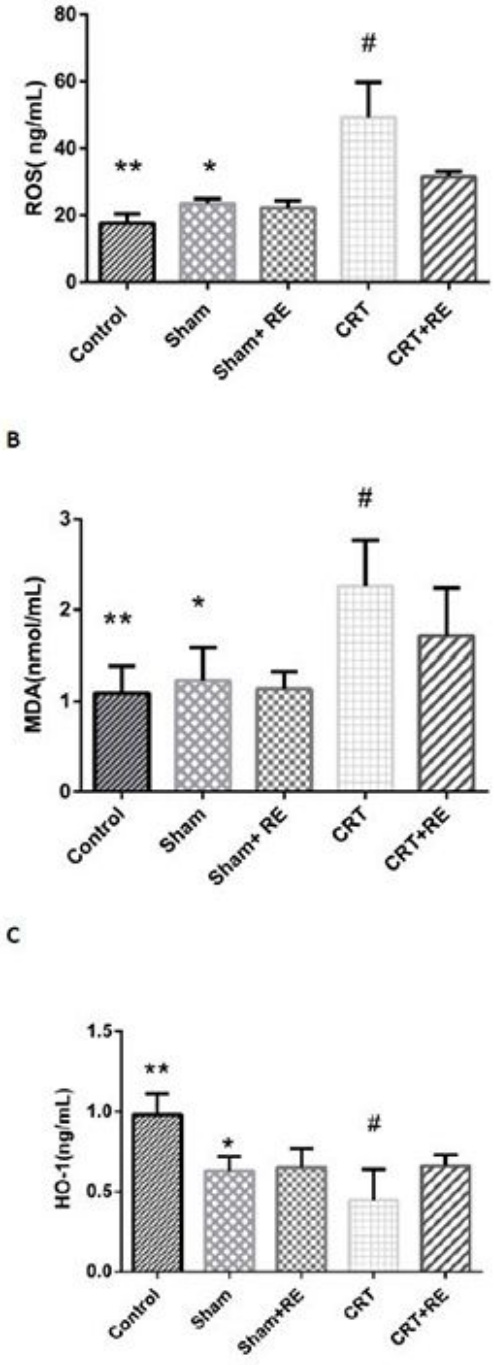


Figure 4

The expression levels of ROS(A), MDA (B) and HO-1(C) in serum of rats. ** p<0.01,VS .CRT ,* P0.05,Vs. Sham+RE,# P <0.01,VS. CRT+ RE.

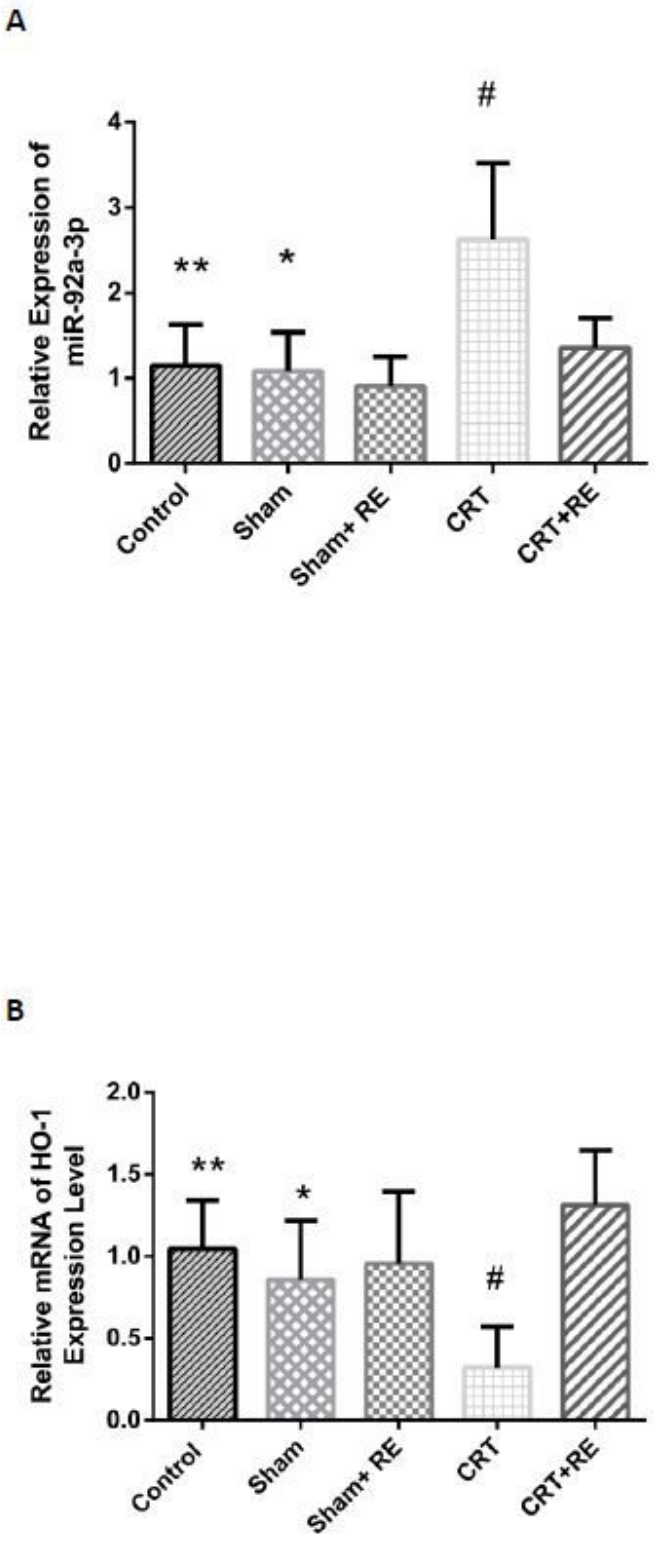


Figure 5

Expression of miR-92a-3p (A) and HO-1 (B) mRNA in rat tissues **p < 0.01,VS. CRT groups, *P0.05, VS. Sham+RE,# p < 0.01,VS CRT+ RE groups.

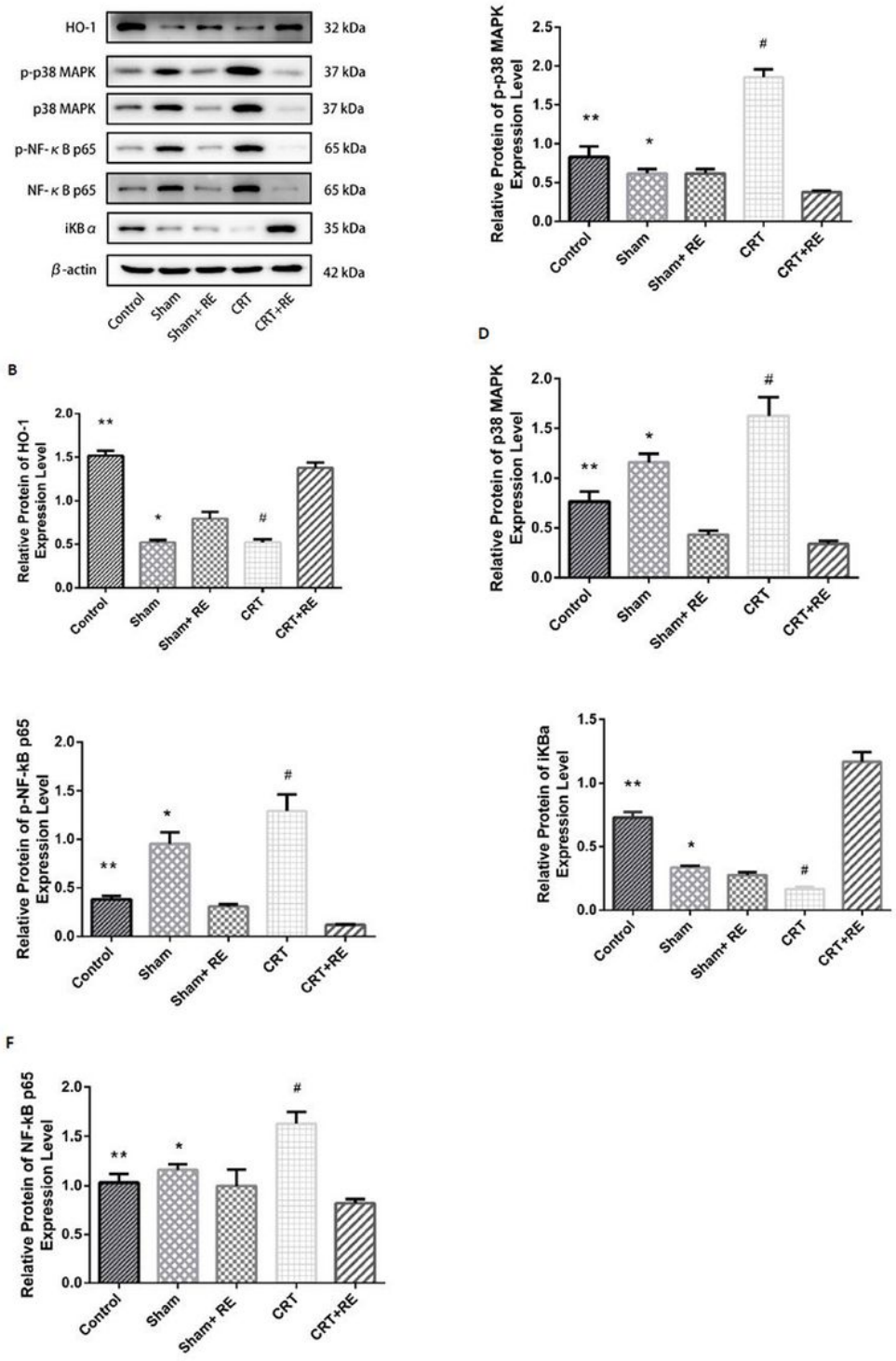


Figure 6

A Western blot image of HO-1、p-p38MAPK、p38 MAPK, P-NF-κB p65、NF-κB p65、IkBa . B-G shows the analysis of protein expression of HO-1、p-p38MAPK、p38 MAPK, P-NF-κB p65、NF-κB p65、IκBa。 **p < 0.01,VS. CRT groups。#p < 0.01,VS. CRT+ RE groups.B-E HO-1、p38 MAPK、p-p38 MAPK and p-NF-kB p65,*p< 0.01,VS.Sham+RE groups.