Resistance Exercise Induces Heme Oxygenase-1 Expression to Promote Macrophage Polarization and Accelerate Catheter-related Thrombolysis and Recanalization in Rats

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Research

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Abstract

Introduction

Catheter-related thrombosis (CRT) is a major complication of central venous catheters. Resistance exercise to prevent CRT formation has been demonstrated, but there are few studies of CRT dissolution and recanalization. In this study, we focused on resistance exercise, heme oxygenase-1 (HO-1), and macrophage polarization to investigate the mechanism of resistance exercise accelerating thrombolysis and recanalization, providing a theoretical basis for resistance exercise adjuvant therapy of CRT.

Methods

A rat CRT model was established and Intervened with resistance exercise and injection of HO-1 agonist cobalt protoporphyrin (COPP) and the inhibitor tin protoporphyrin IX(SnPP).Thrombolysis and recanalization were observed by hematoxylin and eosin(H&E). Neovascularization was detected by immunohistochemical CD31 staining. The levels of HO-1, IL-6, and IL-10 in the rat sera were determined by enzyme-linked immunosorbent assay (ELISA). The relative expression levels of HO-1, Arg-1, HO-1, CD206, and CD80 mRNA were detected by quantitative polymerase chain reaction (qPCR). The colocalization expression of HO-1 with CD206 and CD86 were detected by immunofluorescence.

Results

Resistance exercise for 28 days could induce HO-1 expression, decrease IL-6 level, increase IL-10 level, increase Arg-1 and CD206 expression, decrease INOS and CD80 expression, and increase CD31 expression, and the co-localization expression of HO-1 and CD206 was significantly higher than that of HO-1 and CD86.

Conclusion

After 28 days of resistance exercise, mechanical stimulation can mediate the expression of HO-1 in macrophages, promote the polarization of M2 macrophages, exert the effects of anti-inflammation and promoting angiogenesis, and accelerate the dissolution and recanalization of CRT.

1. Introduction

Central venous catheter (CVC) is a kind of intravascular catheter whose tip is located in the deep vein of neck, chest or abdomen,while using CVC brings convenience, the complications caused by CRT can not be ignored, especially the formation of CRT [1,2]. Some studies have shown that the incidence of asymptomatic CRT monitored by venography is as high as 41%, while the incidence of symptomatic CRT is only 1% -5% [3,4]. It can be seen that CRT has the characteristics of "high risk, multiple occurrence and concealment", which not only leads to catheter dysfunction and disruption of treatment, but also increases patient morbidity and mortality, hospital stay and medical expenses. Furthermore,the purpose of anticoagulant therapy is to prevent the further development of thrombus and to slow the natural recanalization of thrombus, but it does not accelerate the process of thrombolysis[5]. Thrombolytic therapy can rapidly remove thrombus, but it is limited to fresh thrombus. In addition,anticoagulation, thrombolytic therapy, and treatment timing still lack thorough evaluation of safety and effectiveness [2, 6].

Resistance exercise not only improves vascular endothelial function and blood flow velocity, but also inhibits the adverse effects of anticoagulants such as warfarin and heparin and prevents the formation of CRT[7]. As a primary form of prevention,resistance exercise is safe, noninvasive, straightforward to

learn, economical for patients, and easy to popularize;it has been recommended by clinical guidelines for daily prevention and rehabilitation training of cardiovascular diseases [8] . The expression of exercise-induced HO-1 has been confirmed by different studies. Different studies have shown that the expression of HO-1 has different dependence on exercise time and exercise intensity, but all show that exercise induction is closely related to the expression of HO-1 [9,10]. Therefore, the expression of HO-1 induced by resistance exercise may be mediated by hemodynamic stimulation and play a role in vascular protection. In recent years, some scholars have discussed the role of walking exercise in the treatment of DVT and found that exercise can change the shear stress of vein wall and thrombus wall, thus promoting the therapeutic effect of DVT, but the specific regulation mechanism is still unclear [11]. Focusing on exercise-mediated biomechanical activity will bring new ideas for the development of non-pharmacological adjuvant therapy for CRT.

Macrophages play an important role in normal human development, angiogenesis, tissue repair and immune response, and the dynamic process of their functional changes is called polarization[12]. Macrophages have a high degree of heterogeneity and plasticity, and can be polarized into pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages under different microenvironments[13]. M1/M2 phenotypic macrophages have the opposite effect on angiogenesis. M1 macrophages inhibit cell proliferation and restrict angiogenesis, while M2 macrophages can promote angiogenesis through fibrin growth factor and placental growth factor signal pathways. Thus it can be seen that regulating the polarization balance of M1 / M2 macrophages, regulating inflammation and angiogenesis will become a new focus to accelerate thrombolysis and recanalization.

In this study, we found that resistance exercise for 28 days could induce the expression of HO-1, inhibit inflammation and promote the polarization of macrophages. By exploring the effect and mechanism of resistance exercise in accelerating thrombolysis and recanalization, it can provide evidence support and theoretical basis for the adjuvant therapy of CRT.

2. Material And Methods

2.1. Animals

One hundred and forty-four male Sprague-Dawley rats (age:7~8 weeks,weight:200 ~ 250 g) were provided by Experimental Animal Center, Guangxi Medical University, and the ex-perimental animal breeding license number was SCXK Gui 2020–0003.The feeding environment has a room temperature of 20-25 ℃, humidity of 60%-70%, light / dark cycle of 12 hours, good ventilation and light, and rats are free to eat and drink. This experiment strictly follows the 3R principle of experimental animals and has been examined by the Experimental Animal Ethics Committee of Guangxi Medical University (approval number: 202008006).

2.2. Rat model of catheter-related thrombosis

According to the previous experimental method [15], the rats were anesthetized by intraperitoneal injection of 3% pentobarbital sodium,and after the anesthesia was satisfied, the skin was prepared, skin preparation, disinfection and towel laying were performed on the right neck, 1~2cm was cut longitudinally along the right 0.5cm of the trachea, the right external jugular vein was bluntly separated, the distal end of the free vein was ligated with sutures, the vein was cut and an oblique incision was cut under the ligation point, and the catheter was placed into the superior vena cava. After the return of blood from normal saline, the catheter was ligated and fixed, the end of the catheter was closed with a plug, the wound was sutured, and the catheter was routinely sterilized.The rats were warm and resuscitated during anesthesia.After the anesthesia was awake, the rats were put back into the cage to eat and drink normally.

2.3. Intervention method

Previous studies have found that the incidence of thrombosis was high 7 days after catheterization, and the thrombosis was stable 10 days later [15]. Therefore, in this study, the CRT model of rats diagnosed by B-ultrasound 10 days after catheterization was selected, and the rats were randomly divided into CRT control group (n=36), CRT resistance exercise group (n=36), CRT+COPP group (n = 36) and CRT+SnPP group (n=36).Ten days after catheterization, CRT+COPP group and CRT+SnPP group received intraperitoneal injection of CoPP(c1900;Sigma,St.Louis,MO) and SnPP(sentacruzBiotechnology,Dallas,Texas,USA) 5mg/kg respectively, while CRT group did not do any intervention. The exercise method of CRT resistance exercise group was formulated according to the resistance exercise scheme of Begue [16]. All rats received adaptive training without weight-bearing pre-crawling one week before operation, and the rats that completed adaptive training were included in the experiment, otherwise they were excluded [17]. The rats were trained with tail-bound weight 10 days after catheterization, and the inclination of climbing ladder was set to 85 °. The rats began to climb from the bottom of the ladder, trained 6 days a week, rested for 1 day, 1 group / day, 6 times / group, 2min/ times, interval 20 seconds / time, a total of 4 weeks of training. The weight was 10% of the body weight in the first week, 30% in the second week, 50% in the third week, gradually increased every week, and increased to 70% of the body weight in the fourth week.

2.4. Tissue harvesting

On the 1st, 4th, 7th, 10th, 14th and 28th day after intervention, 6 rats were randomly selected at each time point. After the rats were anesthetized satisfactorily, the blood was collected from the abdominal aorta. The serum was collected by centrifugation and placed in the-80 °refrigerator for ELISA to detect the level of HO-1, IL-6 and IL-10 in the serum. The skin on the catheterization side of the rat was cut and taken from the catheter puncture to the upper segment of the right atrium.Part of the blood vessels were soaked in 10%

formalin and fixed for 24 hours for histological analysis. The other part of the blood vessels were stored in the cryopreservation tube of RNA preservation solution and stored in the refrigerator at-80 ℃ for qPCR detection.

2.5. Thrombolysis and recanalization

The vascular tissue was dehydrated, embedded, sliced, stained with H&E, and the thrombolysis was evaluated by pathologist.The stained sections were collected by digital pathological section scanner (NanoZoomerS60,Hamamatsu,Japan) and analyzed by Image-ProPlus image analysis software. The thrombolysis rate of each group was calculated by the following formula:Thrombolysis rate= [(venous lumen area-thrombus area)/venous lumen area]×100%[18].

2.6. Elisa

The blood collected from the vacuum sampling without anticoagulant was placed with 30min at room temperature, then the serum was obtained by 3000*x*g centrifugation 10min at 4 °C. The concentrations of HO-1 (ml003108,Mlbio, Shanghai, China), IL-6 (ml064292,Mlbio, Shanghai, China) and IL-10 (ml002813,Mlbio, Shanghai, China) were detected by ELISA kit. The operation was carried out strictly according to the instructions of the kit, and the absorbance value was detected by enzyme labeling instrument (SynergyH1,BioTek,USA), and the standard curve was drawn.

2.7. qPCR

The vascular tissue was fully ground with liquid nitrogen, and the total RNA was extracted by adding 1mL Trizol (Invitrogen, CA, USA). After reverse transcribed to cDNA, the PCR was amplified by SYBR Green method, and the compound hole was set up. The experimental results were repeated 3 times.The relative expression levels of the target genes were determined using $2^{-\triangle\triangle}$CT. RPLP1 as internal reference.

The sequences of primers were listed as follows:

HO-1-F:5’-TCTGCAGGGGAGAATCTTGC-3’

HO-1-R:5’-TTGGTGAGGGAAATGTGCCA-3’

Arg-1-F:5’-CCAGTATTCACCCCGGCTAC-3’

Arg-1-R:5’-GTCCTGAAAGTAGCCCTGTCT-3’

INOS-F: 5’-TCCTCAGGCTTGGGTCTTGT-3’

INOS-F:5’-AGAAACTTCCAGGGGCAAGC-3’

MRC1-F:5’-GAGGACTGCGTGGTGATGAA-3’

MRC1-R:5’-CATGCCGTTTCCAGCCTTTC-3’

CD80-F:5’-TGTGGCCCGAGTACAAGAAC-3’

CD80-R:5’-GGGGTAGGGAAGTCAGCTCT-3’

RPLP1-F:5’-AAAGCAGCTGGTGTCAATGTT-3’

RPLP1-R:5’-GCAGATGAGGCTTCCAATGT-3’

2.8. Immunohistochemistry and immunofluorescence staining

Vascular tissues were paraffin-embedded and sectioned, thermally repaired with 0.1% sodium citrate, and anti-CD31 (AF6191, AffinityBiosciences. OH.USA) was incubated overnight at 4℃.The general two-step method kit (PV-9000,ZSGB-Bio,beijing,China) was used to add reaction enhancement solution, seal and incubate the second antibody, DAB chromogenic kit (ZSGB-Bio,beijing,China), hematoxylin re-staining and digital pathological section scanner were used to collect images. Imageproplus image analysis software was used to calculate the average optical density to understand angiogenesis.

For immunofluorescence staining, 0.01M citric acid buffer hot antigen repair, 5% BSA 37°C sealed for 30 min. Incubation first antibody:HO-1 (ab189491,abcam,1:200), incubated overnight at 4 ℃. On the second day, the second antibody Cy3 (goat anti-rabbit, AS007Abdonal,1:200) was added and 30min was incubated at 37 ℃. 0.5%Triton Xmur100 broke the membrane for 10 minutes. 5% BSA blocked 30min, incubated first antibody: CD206 (ab125028,abcam,1:200) and CD86 (bs-1035R,Bioss,1:200), incubated 30min at 37 ℃. Incubation second antibody: fluorescent secondary antibody IgG/488 (goat anti-rabbit, ZF-0511,ZSGB-Bio,beijing,China,1:150) incubated 30min without light, sealed with DAPI anti-fluorescence quenching tablet, observed and photographed by inverted fluorescence microscope (CKX53,OLYMPUS,Japan), and measured thenumber of co-located cells and average fluorescence intensity by Image-ProPlus analysis software.

2.9.Statistical Analysis

SPSS 24.0 and GraphPad Prism 8.0 software were used for data processing. The counting data are expressed by frequency and constituent ratio, and the measurement data are described by mean ±standard deviation (x ±s). Analysis of variance was used to compare between different groups. If the variance was uniform, LSD method was used for pairwise comparison. If the variance was uneven, TamhaneT2 test was used.$P < 0.05$ was consideredstatistically significant.

3. Results

3.1. Basic condition and pathomorphology of CRT in rats

The rats were in a good mental state after operation, the survival rate was 100%, the success rate of catheterization was 100%, and there was no catheter prolapse during the catheter indwelling period. After one day of intervention, it was observed that the formation of CRT tended to be stable, the collagen components of thrombus increased, the number of fibroblasts increased, granulation tissue formed from the vein wall to the center of thrombus, and the thrombus began to organize. Four days later, the cracks in the thrombus were enlarged in the CRT control group and CRT resistance exercise group, and further enlarged in the CRT+COPP group. The thrombus organization was obvious and the recanalization was slow in the CRT+SnPP group. After 7 days, the cracks in the thrombus body were enlarged, the new lumen-like structure was formed and partially recanalized in the CRT control group and CRT resistance exercise group. Partial recanalization in CRT+COPP group and slow recanalization in CRT+SnPP group. Ten days later, granulation tissue formation was observed between thrombus and blood vessel wall in CRT control group and CRT resistance exercise group, and the formation of capillary endothelial cells was covered in thrombus. Red blood cells were formed in CRT+COPP group and partially recanalized. In CRT+SnPP group, thrombus was formed outside the catheter, and a little granulation tissue was formed in the middle. After 14 days, the space between the thrombus and the blood vessel wall was enlarged and partially recanalized in the CRT group, while erythrocytes formed and partially recanalized in the other three groups, but the area was larger in the CRT+SnPP group. After 28 days, new blood vessels formed in the thrombus of CRT control group were anastomosed and recanalized, and the blocked blood vessels were partially reconstructed. Among them, CRT+COPP group and CRT resistance exercise group were close to complete recanalization, and red blood cells were formed in the lumen of CRT+SnPP group, only partially recanalized.

3.2.Thrombolysis rate of each group

The thrombolysis rate of each group increased with time (Table 1). Among them, the thrombolysis rate of CRT+COPP group was higher than that of CRT control group at each time point, while that of CRT+SnPP group was lower than that of CRT control group, indicating that HO-1 plays an important role in CRT thrombolysis. The thrombolysis rate in the CRT resistance exercise group was significantly higher than that in the CRT control group until 28 days after intervention, but there was no significant difference between the CRT+COPP group and the CRT+COPP group ($P > 0.05$). The results showed that after 28 days of resistance exercise, thrombolysis could be accelerated and had the same effect as HO-1 agonist.

Table 1 comparison of thrombolysis rate (%) at different time points in each group (x ±s)

group/Days	Day 1	Day 4	Day 7	Day 10	Day 14	Day 28
CRT control group	16.786±0.023	23.063±0.005	41.392±0.039	51.583±0.030	62.010±0.026	70.681±0.025
CRT resistance exercise group	18.069±0.027 ##	28.154±0.026 ##	40.070±0.043 ##	58.128±0.035 #	65.547±0.011 ##	86.779±0.045**
CRT agonist group	25.824±0.032**	39.449±0.049**	54.040±0.050**	64.390±0.052**	76.198±0.023**	88.883±0.035**
CRT inhibitor group	10.692±0.016*	15.978±0.035*	25.296±0.040**	35.347±0.028**	45.433±0.014**	54.483±0.027**
F	18.491	27.149	22.381	46.501	125.042	67.306
P	0.001	0.000	0.000	0.000	0.000	0.000

P0.05 versus the CRT control group

** P0.01versus the CRT control group

P0.05 versus the CRT agonist group

P0.01versus the CRT agonist group

3.3. Elisa

The levels of HO-1, IL-6, and IL-10 in the rat sera are presented in Fig.2. The results showed that the serum HO-1 and IL-10 levels of the CRT+COPP group increased significantly 1 day after intervention and decreased at 4 days but still maintained an increasing trend, which was significantly higher than that in the CRT control and CRT+Snpp groups.Compared with the other three groups, the CRT+Snpp group showed an overall downward trend at each time point after intervention, and the difference was statistically significant. On the 28th day, the CRT+ resistance-exercise group was significantly higher than that in the CRT group, but no significant difference existed between the CRT+COPP group P=0.957.The serum IL-6 levels of the CRT+COPP group decreased significantly on the 1st day after intervention and maintained a downward trend compared with the CRT and CRT+Snpp groups on the 28th day. Conversely, the CRT+Snpp group showed an overall upward trend compared with the other three groups at each time point after intervention, and the difference was statistically significant. A significant difference existed between CRT+ resistance-exercise and CRT+COPP groups at 1, 4, and 7 days after intervention, but no significant difference existed from 10 days to 28 days. On the 28th day, the CRT+ resistance-exercise group was significantly lower than that of the CRT group. Results showed that after 28 days of resistance exercise, HO-1 expression was induced, the level of inflammatory factor IL-6 was inhibited, and the level of anti-inflammatory factor IL-10 increased.

3.4. qPCR

The HO-1, Arg-1, IONS, CD206 and CD80mRNA expression levels in the venous tissues of rats were detected with qPCR. Figure 3 shows significant upregulation of HO-1, Arg-1, and CD206mRNA expression levels in the CRT+COPP group on the 1st day after intervention and decreased on the 4th day but still maintained an increasing trend. Conversely, the CRT+Snpp group decreased at each time point compared with the other three groups after intervention, and the difference was statistically significant,and the CRT+ resistance-exercise group was significantly higher than that in the CRT group at 28 days, but no significant difference existed between the CRT+COPP groups. The expression of IONS and CD80mRNA decreased significantly on the 1st day after CRT+COPP group intervention and maintained a downward trend compared with the CRT and CRT+Snpp groups on the 28th day, and the difference was statistically significant. Compared with the other three groups, the expression of the CRT+Snpp group showed an overall upward trend at each time point after intervention, and the difference was statistically significant. On the 28th day, the CRT+resistance-exercise group was significantly lower than that in the CRT group, but the difference was not significant compared with the CRT+COPP group. Results

showed that 28 days of resistance exercise could upregulate the expression of Arg-1 and CD206 of HO-1 and M2 phenotypic macrophages, as well as inhibit the expression of the M1 phenotypic macrophage markers IONS and CD80.

3.5.Immunohistochemistry and immunofluorescence staining

CD31 expresses in vascular endothelial space. Compared with CRT control group, the expression of CD31 in CRT+COPP group was significantly higher than that in CRT+SnPP group at each time point, while the expression of CD31 in CRT+SnPP group decreased. After 28 days of intervention, the expression of CD31 in the CRT resistance exercise group was different from that in the CRT control group, but there was no significant difference between the CRT+COPP group and the CRT+COPP group (Fig. 4). The results suggest that the mechanism of HO-1 promoting CRT dissolution may be related to its function of promoting neovascularization.

The results of immunofluorescence showed that on the 1st and 28th day after intervention, the co-localization expression of HO-1 and M2 macrophage marker CD206 in CRT+COPP group was significantly higher than that in HO-1 and M1 macrophage marker CD86, while the result in CRT+SnPP group was opposite to that in CRT+SnPP group. In CRT resistance exercise group, the co-localization expression of M2 macrophage marker CD206 was significantly higher than that of HO-1 and M1 macrophage marker CD86 until 28 days after intervention. The results showed that after 28 days of resistance exercise intervention, the co-localization expression of HO-1 and M2 macrophage marker CD206 was significantly higher than that of HO-1 and M1 macrophage marker CD86. (Fig. 5)

4. Discussion

In this study, we demonstrated that after 28 days of resistance exercise intervention, mechanical stimulation can induce the high expression of HO-1 in macrophages, which can safely and effectively accelerate the dissolution and recanalization of CRT. The results of Elisa and qPCR analysis showed that after the intervention of resistance exercise for 28 days, the inflammatory reaction was inhibited, the expression of M2 macrophage markers Arg-1 and CD206 increased, while the expression of M1 macrophage markers IONS and CD80 decreased. The results of immunofluorescence and immunohistochemistry showed that the expression of HO-1 in M2 macrophages was significantly higher than that in M1 macrophages, and showed that HO-1 could promote thrombolysis and recanalization, and its mechanism was related to its angiogenesis function.

Resistance exercise promotes venous and lymphatic reflux and improves hemodynamics through musculoskeletal contraction. Short-term (4-12 weeks) resistance training can also regulate serum metabonomics, thus promoting anti-atherosclerotic effects and cardiometabolic health[19.20]. There is evidence that exercise can increase the level of HO-1, reduce oxidative stress injury, and exert the function of anti-inflammation and antioxidation[21]. Research found that resistance exercise can reduce the levels of TNF- α, IL-6 and CRP, and increase the level of IL-10, suggesting that exercise training can inhibit inflammatory response [22]. At the same time, exercise can promote the anti-inflammatory phenotype of macrophages.The study found that exercise enhanced the anti-inflammatory / M2 phenotypic polarization of alveolar macrophages,it is suggested that the changes in the number and inflammatory state of alveolar macrophages may be a way of the protective mechanism of exercise on lung injury [23]. Previous studies have shown that resistance exercise can activate skeletal muscle Akt signal, enhance the expression of HO-1 by macrophages, increase M2 macrophages, secrete VEGF, induce angiogenesis, and promote the recovery of blood flow in ischemic lesions [24]. It can be seen that exercise is closely related to the expression of HO-1 and the polarization of macrophages to M2 phenotype. This study showed that resistance exercise intervention for 28 days could up-regulate HO-1 and decrease the level of IL-6, inhibit inflammatory reaction, promote the expression of M2 marker Arg-1 and CD206 in macrophages, and accelerate the dissolution and recanalization of CRT. Therefore, it is speculated that regular exercise may mediate HO-1 expression in macrophages, affect M1/M2 macrophage polarization balance, play a role in promoting angiogenesis, and affect thrombolysis and recanalization to a certain extent through mechanical stimulation.

HO-1 is mainly expressed in macrophages during the occurrence of various diseases,and due to its ability to regulate inflammatory process and tissue damage, the expression of HO-1 in cardiovascular cells is considered to be the protective mechanism in the state of disease[25]. HO-1 can be a marker protein reflecting the oxidative status in M2 macrophages because of its adaptive response to oxidative status, and in inflammation, after inducing HO-1 expression, macrophages show M2 macrophage phenotypic function, while inhibiting M1 macrophage expression; after inhibiting HO-1 expression, M2 macrophage marker expression is decreased and leads to aggravated inflammation.[26,27] HO-1 is highly induced by IL-6, while HO-1 negatively regulates IL-6, which indicates that there is a negative feedback loop between HO-1 and IL-6[28]. While macrophages are a potential source of IL-10, IL-10 and HO-1 are interconnected through a positive feedback loop, IL-10 stimulation can promote STAT3 phosphorylation and gene up-regulation, which

can shift macrophages to the M2 phenotype[29] In this study, the level of IL-6 decreased and the level of IL-10 increased after induction of HO-1. At the same time, the results of immunofluorescence co-expression showed that the expression of M2 macrophages was significantly higher than that of M1 macrophages. It was further explained that 28 days of resistance exercise induced the expression of HO-1, inhibited the inflammatory reaction, promoted the polarization of M2 macrophages and promoted the dissolution and recanalization of CRT. A study shows that monocyte-derived macrophages isolated and cultured from the blood of patients with coronary artery disease highly expressed HO-1 and affected the polarized phenotype of mononuclear macrophages, which was associated with macrophage content accumulation, thinner fibrous cap, and thrombus plaque rupture in atherosclerotic plaques [30]. Other studies have found that activated protein C can up-regulate the expression of HO-1 in macrophages and promote the dissolution of venous thrombosis[31]. It can be seen that HO-1 is closely related to M2 macrophage activation and thrombolysis and recanalization.

Inflammation and angiogenesis are the key mechanisms of thrombolysis and recanalization. The dissolution of venous thrombus is very important for vascular patency, which is similar to the normal wound healing process. It requires a coordination of fibrinolysis, proteolysis, inflammation and angiogenesis[32]. Neovascularization is the key to thrombolysis and recanalization. At the early stage of thrombus organization, the thrombus shrinks from the vascular wall, resulting in the formation of a pocket and crack structure lined by endothelial cells between the main body of the thrombus and the intima of the venous wall, the formation of a new vascular lumen and mutual fusion and expansion, the gradual recovery into a prothrombotic state, so that the blocked vein restores blood flow [33]. As an adhesive stress response protein, CD31 is highly expressed in endothelial cell-cell junction and is a marker of angiogenesis. It can not only maintain the integrity of endothelial cell connection, but also accelerate the repair of vascular permeability barrier after inflammation or thrombosis[34]. In this study, CD31 in vascular tissue was detected and analyzed by immunohistochemistry. The results show that inducing the expression of HO-1 can promote the dissolution and recanalization of CRT, and its mechanism may be related to its function of promoting neovascularization. Other studies have shown that HO-1 could promote the proliferation, mobilization and migration of endothelial progenitor cells (EPCs), and accumulate to the injured area to differentiate into new endothelial cells, thereby promoting vascular repair and enhancing their viability and anti-oxidative stress injury ability, which may be the key mechanism by which HO-1 promotes angiogenesis[35].

Aiming at the controversy and uncertainty of clinical "high-risk, multiple and hidden" CRT and resistance exercise accelerating CRT dissolution and recanalization, this study proved the safety and effectiveness of resistance exercise accelerated CRT dissolution and recanalization from animal experiments, and provided strong evidence and economic, scientific and effective adjuvant therapy for clinical resistance exercise adjuvant therapy of CRT. However, this study also has some limitations, such as the failure to use B-ultrasound to monitor the dissolution and recanalization of rat CRT and to verify the mechanism of dissolution and recanalization from other molecular signal pathways, our research team will continue to study in depth.

Conclusion

Resistance exercise can safely and effectively accelerate the dissolution and recanalization of CRT. By mediating mechanical stimulation, it can induce the high expression of HO-1 in macrophages and enhance the expression of M2 macrophages, so as to inhibit inflammatory reaction, promote neovascularization and accelerate the dissolution and recanalization of CRT.

Abbreviations

CRT: Catheter-related thrombosis; CVC: Central venous catheter;

HO-1: Heme oxygenase-1; COPP :Cobalt protoporphyrin;

SnPP:tin protoporphyrin IX; H&E:Hematoxylin and eosin;

ELISA: Enzyme-linked immunosorbent assay;qPCR: Quantitative polymerase chain reaction

Declarations

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Authors' contributions

JW,HZ and YY conceived the study and designed the experiment. XH, JW, QJ and CW made substantial contributions to the experiment and acquisition of data. JW analyzed data and wrote the manuscript. LY 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 and approved the final version of the manuscript.

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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 (approval number: 202008006). 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 that they have no competing interests.

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Figures

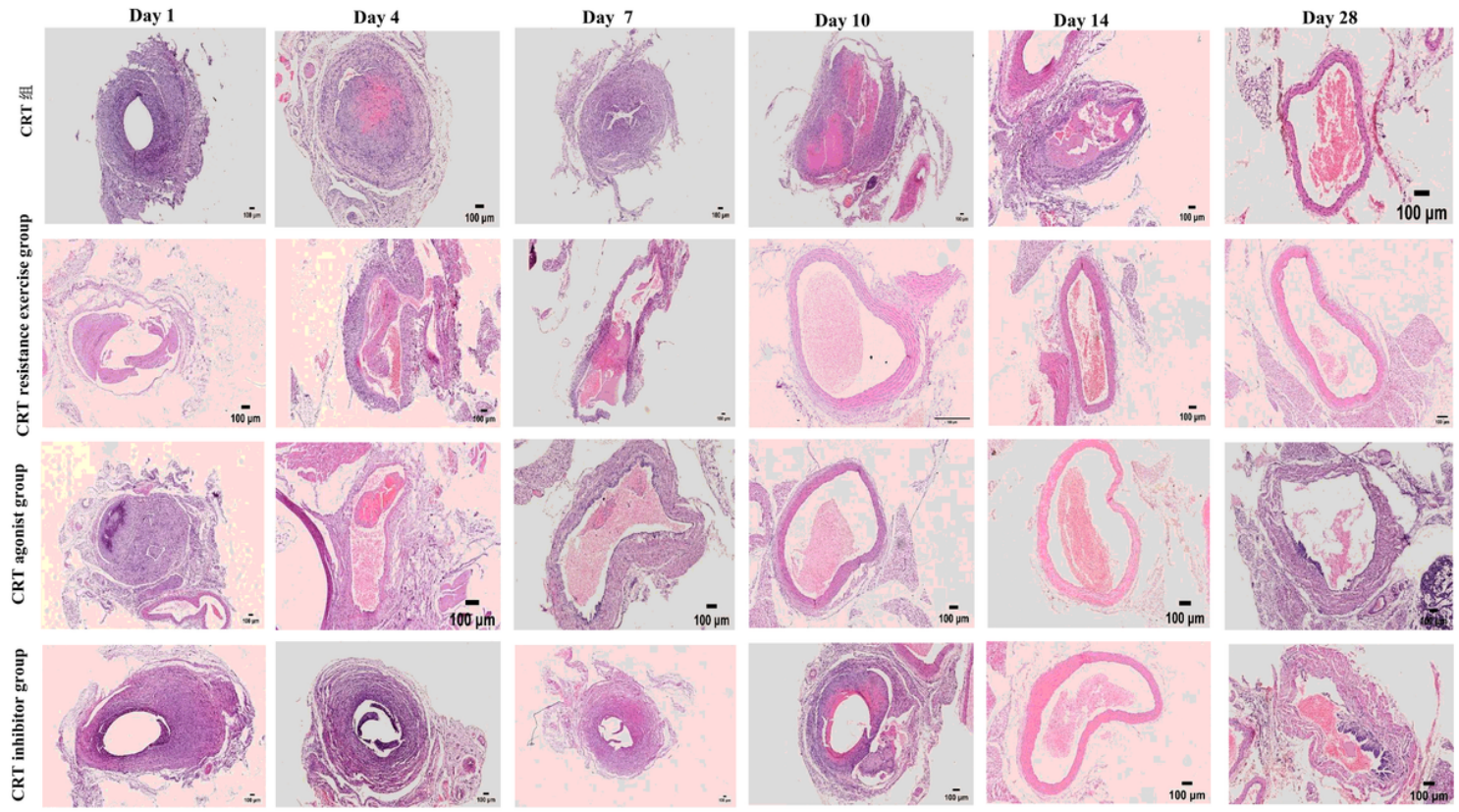

Figure 1

HE staining of vascular tissue of rats in each group at each time point (50 ×)

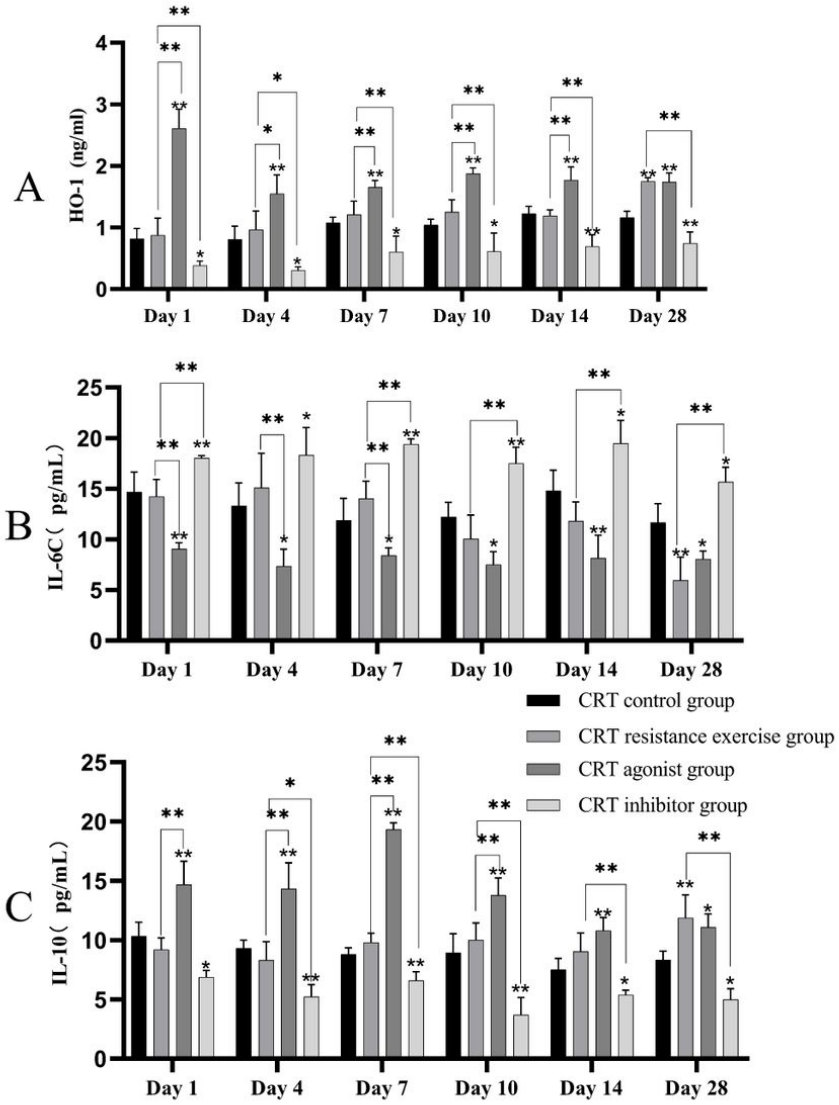

Figure 2

The levels of HO-1 in the rat sera at different time points in each group. (A) The levels of IL-6 in the rat sera at different time points in each group. (B) The levels of IL-10 in the rat sera at different time points in each group. (C)

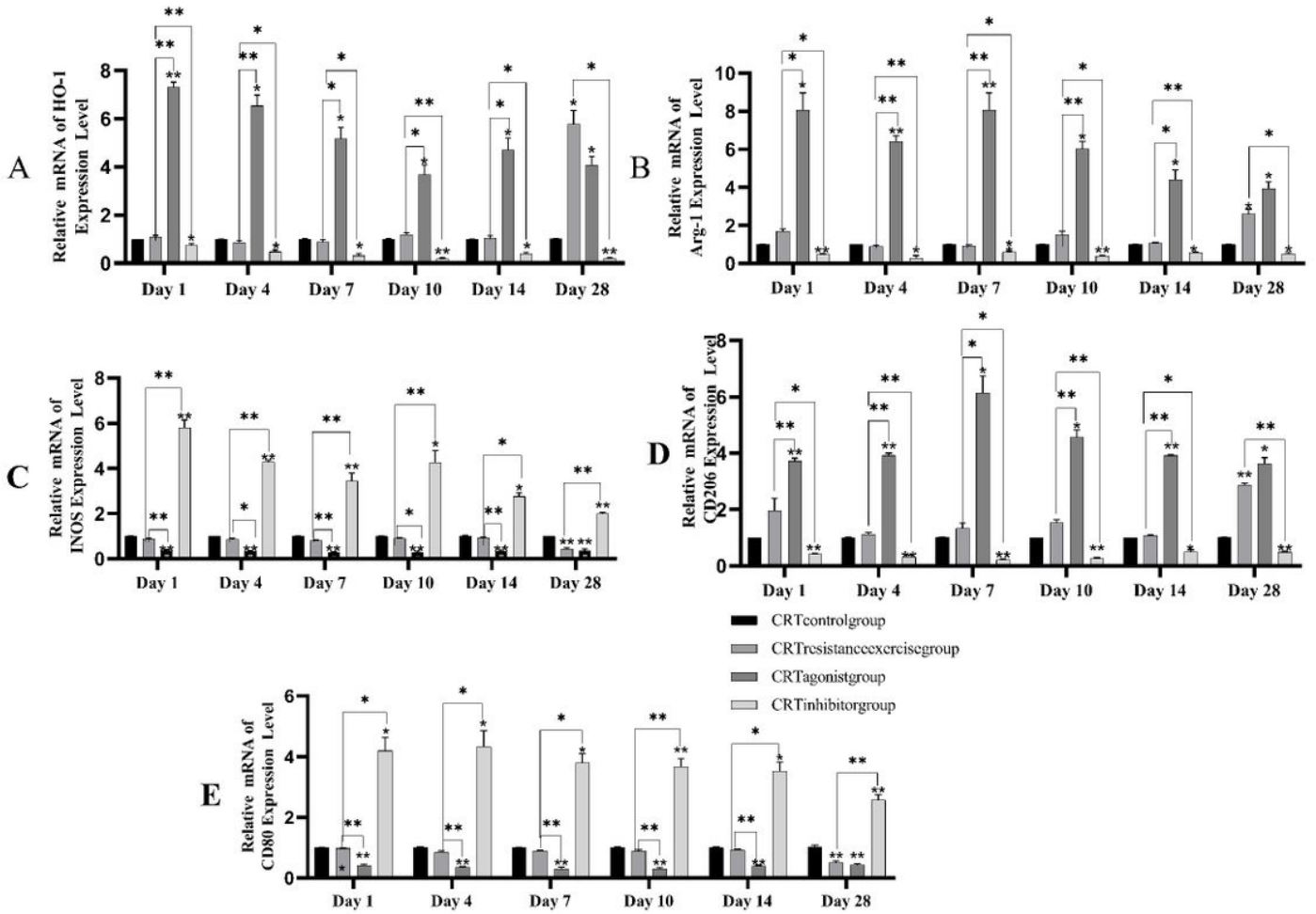

Figure 3

HO-1mRNA expression at different time points in each group (A) Arg-1mRNA expression at different time points in each group (B) IONSmRNA expression at different time points in each group (C) CD206mRNA expression at different time points in each group (D) CD80mRNA expression at different time points in each group (E)

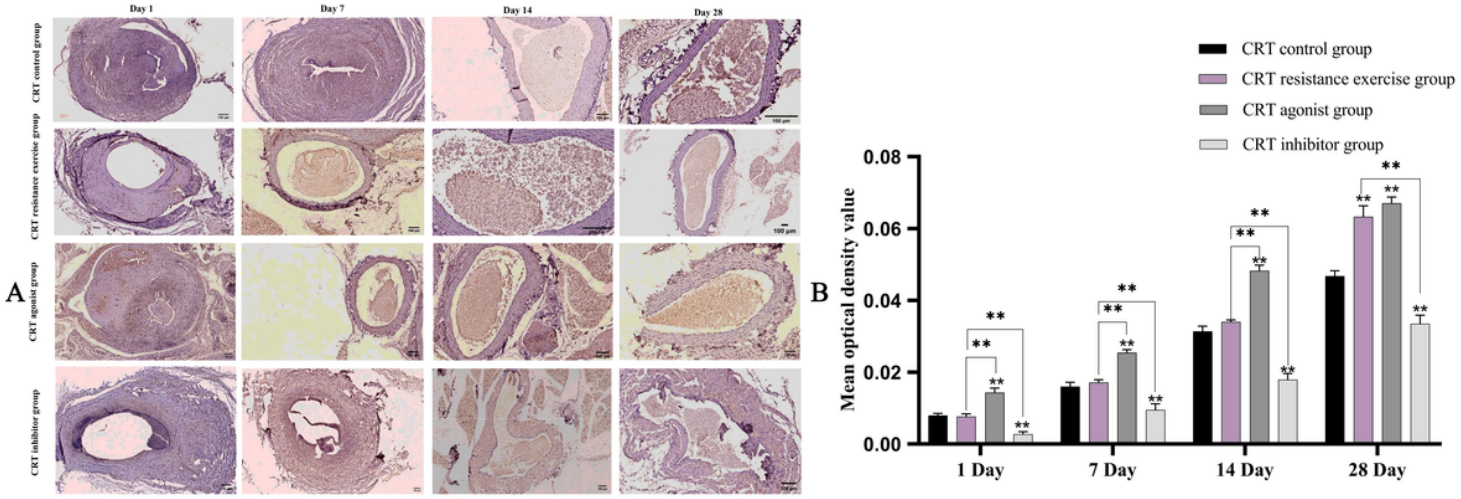

Figure 4

CD31 immunohistochemical staining at 1, 7, 14 and 28 days after intervention (100 ×) (A) the average optical density at 1, 7, 14 and 28 days after intervention in each group. (B)

Figure 5

The co-localization expression of HO-1 and CD206 in each group was intervened for 1 day. (A) The expression of HO-1 and CD86 in each group was intervened for 1 day. (B) The co-localized expression of HO-1 and CD206 in each group for 28 days. (C)The expression of HO-1 and CD86 in each group was intervened for 28 days. (D) The number of co-localized cells and immunofluorescence intensity of HO-1&CD206 and HO-1&CD86 in each group after intervention for 1 day and 28 days. (E)