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Original Paper

Renal Denervation Effects on Myocardial Fibrosis and Ventricular Arrhythmias in Rats with Ischemic Cardiomyopathy

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Key Words
Renal Denervation • Myocardial Infarction • Ventricular Arrhythmia • Fibrosis • Connexin

Abstract
Background/Aims: To investigate the impact of renal denervation (RDN) on myocardial fibrosis and ventricular arrhythmias (VAs) in rats with ischemic cardiomyopathy. ***Methods:*** An ischemic cardiomyopathy model was reproduced with myocardial infarction (MI) in adult Sprague–Dawley male rats. The RDN/Sham-RDN procedure was performed at 2 weeks after MI. Sham-MI and sham-RDN rats served as the control group. At 4 weeks after RDN, programmed electrical stimulation (PES) was used to induce VAs, including ventricular tachycardia and ventricular fibrillation, in all 3 groups (MI+RDN, MI, and control groups). At the end of PES, heart and kidney samples were harvested. Immunofluorescence labeling was used to investigate the distribution of connexin 43 (Cx43) in the infarcted border zone. Masson's trichrome stain was adopted to determine the degree of cardiac fibrosis. Western blotting was performed to identify the expression of transforming growth factor beta 1 (TGF-β1), α-smooth muscle actin (α-SMA), and Cx43. An enzyme-linked immunosorbent assay (ELISA) was used to detect the serum levels of B-type natriuretic peptide (BNP) and the amino-terminal propeptides of type I and III collagen (PINP and PIIINP, respectively) and the expression level of renal norepinephrine. ***Results:*** Compared with the MI group, RDN significantly decreased the inducibility of VAs (MI+RDN 3/8 rats vs. MI 8/9 rats, $P < 0.05$; control 1/8 rats) with PES, reduced myocardial fibrosis estimated by collagen volume fraction (MI+RDN 31.10 ± 3.97% vs. MI 54.80 ± 16.39%, $P < 0.001$; control 4.41 ± 0.92%), suppressed TGF-β1 ($P < 0.01$) and α-SMA ($P < 0.001$) levels, and attenuated both PINP (MI+RDN 41.44 ± 10.10 ng/mL vs. MI 95.49 ± 24.83 ng/mL, $P < 0.001$; control 11.90 ± 4.96 ng/mL) and PIIINP (MI+RDN 82.12 ± 30.79 ng/mL vs. MI 124.60 ± 26.64 ng/mL, $P < 0.05$; control 64.69 ± 23.84 ng/mL) levels. Moreover, RDN reversed the abnormal myocardial distribution of Cx43 and its reduction by MI damage ($P < 0.01$). ***Conclusions:*** RDN reduced myocardial fibrosis and suppressed VAs in a rat model of ischemic cardiomyopathy.

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Introduction

The cause of death varies at different times in ischemic cardiomyopathy after myocardial infarction (MI). Fatal ventricular arrhythmias (VAs) resulting from ischemia lead to sudden death in the acute state of MI. Over time, cardiac remodeling induces VAs leading to death [1]. Hence, any approaches to improve structural remodeling might also have potential antiarrhythmic effects in ischemic cardiomyopathy.

Renal denervation (RDN), which is a novel technique originally used for treating resistant hypertension [2], was shown to exert beneficial effects on multi-organ fibrosis in our previous study [3]. RDN has also been reported to attenuate cardiac remodeling and preserve cardiac function in several studies [4, 5]. In addition, with respect to VAs after MI, Linz et al. reported that RDN suppressed VAs during acute ischemia events [6], while Nicholas et al. recently demonstrated its role in reducing post-infarction VAs [7]. However, little is known of the mechanisms underlying these phenomena. Thus, the purpose of the present study was to investigate the effects of RDN on myocardial fibrosis and electrophysiology in a rat model of MI-induced ischemic cardiomyopathy and to explore the possible underlying mechanisms.

Materials and Methods

Animal care

Male Sprague–Dawley rats (200-220 g) were obtained from Nanjing Medical University Laboratory Animal Center. All experimental procedures were approved by the Ethics Committee of Nanjing Medical University. This study conformed to institutional guidelines for the care and use of experimental animals.

Induction of an ischemic cardiomyopathy model

An ischemic cardiomyopathy model was generated with MI by ligating the left anterior descending coronary artery, as described previously [8, 9]. In brief, the rats were anesthetized by 2% pentobarbital sodium (40 mg/kg) before they were endotracheally intubated and mechanically ventilated. The pericardium was opened through a thoracotomy in order to ligate the left anterior descending coronary artery with a 7–0 silk suture, which was approximately 2 mm below the tip of the left atrium. After ligation, the surface of the heart turned white immediately, and the thorax was closed. The rats were placed in a 26°C environment to help them regain consciousness. The rats were given pethidine (40 mg/day) and penicillin (400, 000 U/day) intramuscularly to relieve pain and prevent infection for 3 days. The animals in the control group were subjected to a similar procedure but without coronary artery ligation. All MI procedures were performed by the same operators.

RDN

At 2 weeks after MI, bilateral RDN was conducted in the MI+RDN group, as described previously [10]. Briefly, a vertical back incision was performed under general anesthesia with 2% pentobarbital sodium (40 mg/kg). All of the visible nerves were then cut. In addition, the remaining nerves were destroyed by painting around the renal arteries with a solution of 20% phenol in ethanol. Finally, the arteries were washed with normal saline. The same procedures were performed on the MI group rats, except for damaging the nerves. The RDN procedure was conducted by the same investigators.

Echocardiography

Cardiac function was examined by echocardiography [11]. Echocardiography was performed in rats anesthetized with isoflurane at 2 weeks (before RDN) and repeated at 6 weeks after MI. A Vevo2100 (VisualSonics, Canada) system equipped with an MS-250, 16.0-21.0 MHz imaging transducer was applied for echocardiography. Heart rate (HR) was recorded at the same time. The investigators were blinded to the treatment of the rats.

Ventricular programmed electric stimulation

Electrophysiological studies *in vivo* were performed at 4 weeks after RDN [12]. Electrocardiography was administered with 3 needle electrodes attached to the right upper limb and legs. Programmed electrical stimulation (PES) was used to stimulate the left ventricular apex of the heart through a bipolar electrode to investigate the incidence of VAs; the threshold potential for stable pacing was gained at a cycle length of 140 ms. Pacing was started with twice as much as the threshold and a cycle length of 140 ms, which was the interval of 8 stimuli (S1). An extra stimulus (S2) was applied until it failed to induce ventricular depolarization, while the interval (S1-S2) was shortened progressively by 10 ms. The operators were blinded to the treatment of the rats.

Immunofluorescence labeling

Immunofluorescence labeling was used to investigate the distribution of connexin 43 (Cx43) in the infarcted border zone (IBZ) [13]. The specimens were evaluated under a fluorescence microscope (Nikon, Japan).

Histopathological analysis

As soon as each rat was sacrificed, the heart and kidneys were removed. The heart samples were cut transversely into 2 parts: one was fixed with 4% paraformaldehyde and then embedded in paraffin, while the other and the kidney samples were stored at -80℃. Sections cut from heart paraffin blocks were stained with Masson's trichrome to determine the degree of cardiac fibrosis with the collagen volume fraction (CVF), as described previously. At least 5 microscopic fields (×100) were selected randomly to analyze CVF using Image-Pro Plus 6.0 software.

Western blotting

Western blotting was performed to identify the expression of transforming growth factor beta 1 (TGF-β1), α-smooth muscle actin (α-SMA), and Cx43 (Proteintech, China). Ventricle samples were lysed in lysis buffer (Beyotime, China), and the protein concentrations were determined using the BCA method, as described previously. Glyceraldehyde-3-phosphate dehydrogenase (Proteintech, China) was used to normalize protein levels.

Enzyme-linked immunosorbent assay

Plasma samples used for an enzyme-linked immunosorbent assay (ELISA) were obtained by centrifuging blood. Kidney samples were also homogenized and centrifuged to obtain supernatant. All samples were centrifuged at 3000 rpm. for 10 min at 4°C. The serum levels of B-type natriuretic peptide (BNP) and amino-terminal pro-peptides of type I and III collagen (PINP and PIIINP, respectively) and the expression level of renal norepinephrine (NE) in the supernatant of kidney samples were tested using an ELISA kit (Elabscience Biotechnology, China). All procedures were performed according to the manufacturer's instructions

Statistical analysis

Continuous variables are presented as mean ± standard deviation. For multiple comparisons, if the variance test was homogeneous, the data were analyzed by one-way analysis of variance followed by least significant difference test, or else Dunnett's 3T test was adopted. To compare the inducibility of VAs, Fisher's exact test was applied. SPSS 16.0 software (SPSS, Inc., USA) was used to analyze the data, and P values < 0.05 were considered significant.

Results

Effectiveness of RDN

Renal NE levels were tested at 4 weeks after RDN in 3 groups (Fig. 1). Compared with the MI group, a significant decrease of NE was found in the MI+RDN group (MI+RDN 0.0213 ± 0.0034 ng/mg vs. MI 0.0324 ± 0.0024 ng/mg, P < 0.001), which proved the effectiveness of the RDN procedure.

Cardiac function and HR at 2 weeks

At 2 weeks after MI, 8 rats in the control group and 17 rats treated with MI had survived. The 17 surviving MI rats were divided randomly into the MI group (n = 9) and MI+RDN group (n = 8). Echocardiography was performed to measure cardiac structure and function. Left ventricular end systolic diameter (LVDs, all MI 7.142 ± 0.694 mm vs. control 5.126 ± 0.267 mm, P < 0.001) and left ventricular end diastolic diameter (LVDd, all MI 9.284 ± 0.682 mm vs. control 8.491 ± 0.362 mm, P < 0.01) were increased in the 17 MI rats compared with the control rats (Fig. 2a-b and Table 1). Conversely, the ejection fraction (EF, all MI 44.33 ± 4.75% vs. control 68.00 ± 3.58%, P < 0.001), fractional shortening (FS, all MI 23.15 ± 2.94% vs. control 39.57 ± 2.99%, P < 0.001), interventricular septal thickness in systole (IVSs, all MI 1.795 ± 0.515 mm vs. control 2.816 ± 0.117 mm, P < 0.001), and interventricular septal thickness in diastole (IVSd, all MI 1.295 ± 0.251 mm vs. control 1.736 ± 0.072 mm, P < 0.001) (Fig. 2c-f and Table 1) were decreased significantly in the 17 MI rats in comparison with the control rats, indicating the successful generation of the MI model. Furthermore, at this time, the rats that suffered MI had a higher HR than those that did not (all MI 363.5 ± 33.9 bpm vs. control 323.6 ± 15.8 bpm, P < 0.01) (Fig. 2g).

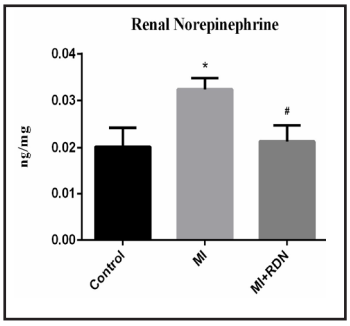

Fig. 1. ELISA assay of renal norepinephrine. Renal norepinephrine level was significantly reduced through RDN compared with MI rats. (n = 8, 9, 8 in Control, MI and MI+RDN group, respectively. Data were mean ± SD. *P<0.05 vs. Control group; # P<0.05 vs. MI group).

Table 1. Echocardiographic characteristics at Week 2 and 6. EF = ejection fraction; FS = fractional shortening; IVSd = interventricular septal thickness in diastole; IVSs = interventricular septal thickness in systole; LVDd= left ventricular end diastolic diameter; LVDs = left ventricular end systolic diameter; LAD = left atrial diameter

Group	Week	EF(100%)	FS(100%)	IVSd(mm)	IVSs(mm)	LVDd(mm)	LVDs(mm)	LAD(mm)
Control	2	68.00±3.58	39.57±2.99	1.74±0.07	2.82±0.12	8.49±0.36	5.13±0.27	4.87±0.45
	6	67.37±4.81	39.14±3.85	1.75±0.06	2.76±0.12	8.56±0.34	5.21±0.42	5.05±0.49
MI	2	44.30±4.87	23.15±2.94	1.21±0.29	1.70±0.64	9.31±0.80	7.16±0.77	4.85±0.70
	6	43.40±7.52	22.81±4.49	1.18±0.39	1.65±0.61	10.24±1.10	7.93±1.17	4.92±0.99
MI+RDN	2	44.36±4.94	23.18±2.93	1.39±0.17	1.90±0.34	9.25±0.57	7.12±0.65	4.87±0.39
	6	56.91±4.44	31.43±2.92	1.71±0.47	2.58±0.60	9.44±1.04	6.49±0.89	4.53±0.35

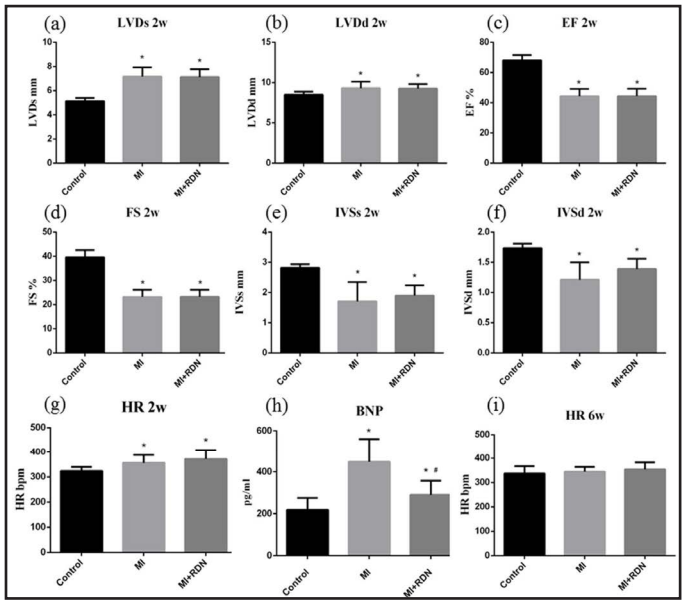

Fig. 2. MI resulted in cardiac dysfunction at 2 weeks. Echocardiography evaluation of (a) LVDs (b) LVDd (c) EF (d) FS (e) IVSs (f) IVSd. The heart rate at 2 weeks (g) and 6 weeks (i). ELISA assay of plasma BNP at 6 weeks (h). (n = 8, 9, 8 in Control, MI and MI+RDN group, Data were mean ± SD. *P<0.05 vs. Control group; # P<0.05 vs. MI group).LVDs = left ventricular end systolic diameter; LVDd= left ventricular end diastolic diameter; EF = ejection fraction; FS = fractional shortening; IVSs = interventricular septal thickness in systole; IVSd = interventricular septal thickness in diastole;

Cardiac function and HR at 6 weeks

At 6 weeks after MI, the changes in LVDs (MI+RDN 6.488 ± 0.888 mm vs. MI 7.930 ± 1.169 mm, P < 0.01), LVDd (MI+RDN 9.441 ± 1.043 mm vs. MI 10.240 ± 1.097 mm, P < 0.05), EF (MI+RDN 56.91 ± 4.44% vs. MI 43.40 ± 7.52%, P < 0.001), FS (MI+RDN 31.43 ± 2.92% vs. MI 22.81 ± 4.49%, P < 0.001), IVSs (MI+RDN 2.581 ± 0.598 mm vs. MI 1.652 ± 0.607 mm, P < 0.01), and IVSd (MI+RDN 1.713 ± 0.475 mm vs. MI 1.180 ± 0.391 mm, P < 0.05) were attenuated significantly in the MI+RDN group compared to the MI group (Fig. 3a-g and Table 1). In addition, there was no difference in LVDd (MI+RDN 9.441 ± 1.043 mm vs. control 8.560 ± 0.339 mm, P > 0.05), IVSs (MI+RDN 2.581 ± 0.598 mm vs. control 2.756 ± 0.119 mm, P > 0.05), and IVSd (MI+RDN 1.713 ± 0.475 mm vs. control 1.753 ± 0.064 mm, P > 0.05) between the MI+RDN group and the control group. In accordance with the echocardiography findings, the plasma BNP level was significantly higher in the MI group than in the MI+RDN group (MI+RDN 289.9 ± 67.18 pg/mL vs. MI 447.8 ± 112.3 pg/mL, P < 0.001) (Fig. 2h). However, no difference was found in HR among the 3 groups at this time point (control 336.9 ± 30.6 bpm vs. MI+RDN 353.5 ± 29.8 bpm vs. MI 344.2 ± 19.6 bpm, P > 0.05) (Fig. 2i).

Effects of RDN on VAs

The outcomes of ventricular PES are shown in Fig. 4a-c. VAs, including ventricular tachycardia and ventricular fibrillation, were induced more easily in the MI group than in the MI+RDN group. In detail, induced VAs were present in 8/9 rats from the MI group and 3/8 rats from the MI+RDN group (P < 0.05), while only 1/8 rats from the control group suffered such consequences (Fig. 4d).

Effects of RDN on fibrosis

CVF was used to estimate the degree of cardiac fibrosis through Masson's staining. Obvious fibrosis was observed in the MI-treated rats compared with the control rats, while the RDN procedure significantly attenuated CVF (MI+RDN 31.10 ± 3.97% vs. MI 54.80 ± 16.39%, P < 0.001) (Fig. 5). The expression of TGF-β1 and α-SMA, both of which are fibrosis-related factors, was significantly up-regulated in the heart by coronary occlusion. However, in the MI+RDN group, these changes were partially repaired (Fig. 6a). In accordance with this, the plasma levels of PⅠNP (MI+RDN 41.44 ± 10.10 ng/mL vs. MI 95.49 ± 24.83 ng/mL, P < 0.001) and PⅢNP (MI+RDN 82.12 ± 30.79 ng/mL vs. MI 124.60 ± 26.64 ng/mL, P < 0.05) were significantly decreased in the MI+RDN group compared with the MI group (Fig. 6b).

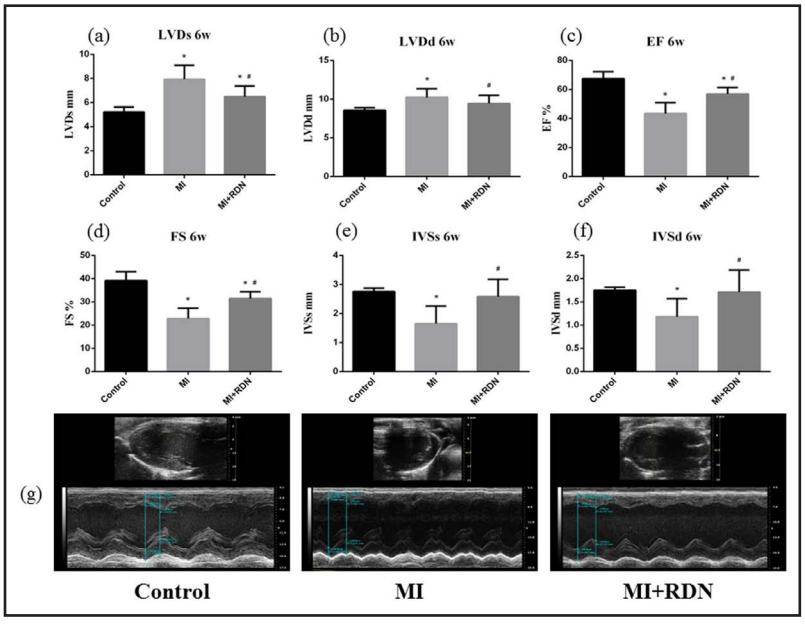

Fig. 3. RDN partly restored cardiac dysfunction at 6 weeks. Echocardiography assessment of (a) LVDs (b) LVDd (c) EF (d) FS (e) IVSs (f) IVSd. Representative tracings of echocardiography (g). (n = 8, 9, 8 in Control, MI and MI+RDN group, Data were mean ± SD. *P<0.05 vs. Control group; # P<0.05 vs. MI group).

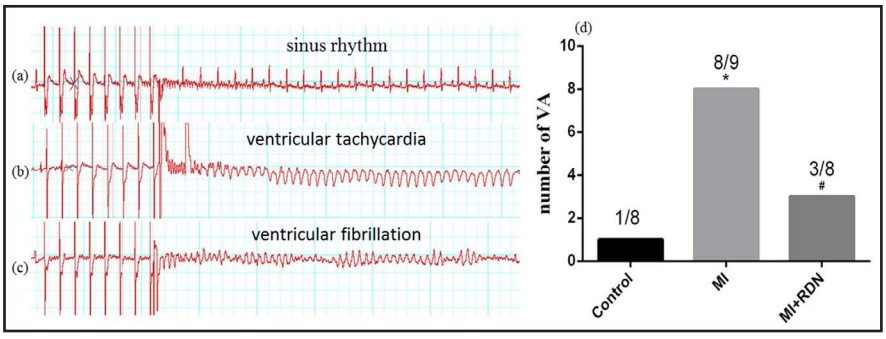

Fig. 4. RDN significantly reduced the inducibility of ventricular arrhythmias.Representative ECG of electrical stimulation, including sinus rhythm (a), ventricular tachycardia (b) and ventricular fibrillation (c). Ventricular arrhythmias were more easily induced in MI group rather than in MI+RDN group (d). (n = 8, 9, 8 in Control, MI and MI+RDN group, *P<0.05 vs. Control group; # P<0.05 vs. MI group).

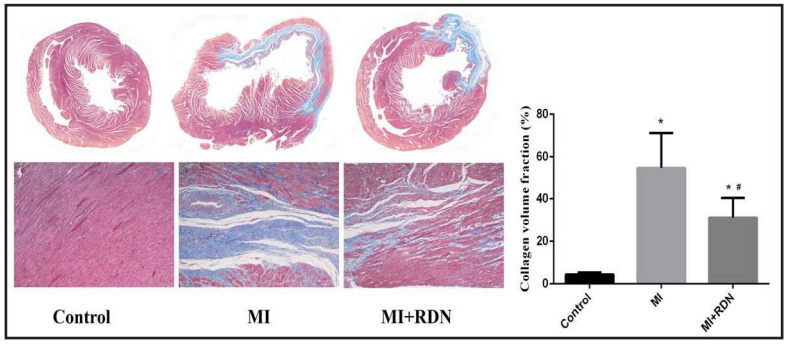

Fig. 5. RDN significantly attenuated cardiac fibrosis. Representative ventricular fibrosis (in blue) by Masson's staining of samples from the control, MI, and MI+RDN groups. Quantitative analysis suggested that CVF in MI+RDN group was significantly lower than that in MI group. (n = 8, 9, 8 in Control, MI and MI+RDN group, Data were mean ± SD. *P<0.05 vs. Control group; # P<0.05 vs. MI group).

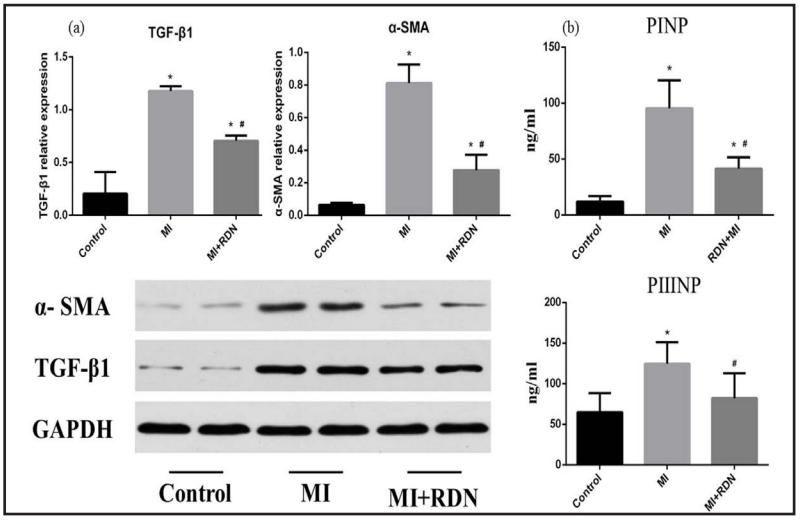

Fig. 6. RDN lowered the expression of TGF-β1, α-SMA, P Ⅰ NP and PⅢNP.Quantitative analysis and representative protein expression of TGF-β1 and α-SMA in heart by Western blot (a). Quantitative analysis of the plasma levels of P Ⅰ NP and PⅢNP by Elisa assay (b). (n = 8, 9, 8 in Control, MI and MI+RDN group, Data were mean ± SD. *P<0.05 vs. Control group; # P<0.05 vs. MI group).

Effects of RDN on Cx43

Immunofluorescence analysis was chosen to evaluate the localization of Cx43. In the IBZ, the distribution of Cx43 was disrupted. This disruption of Cx43 localization was attenuated in the IBZ by the RDN procedure (Fig. 7a). The protein level of Cx43 was examined through western blotting. Compared with the control group, the relative expression of Cx43 was significantly reduced in the MI group; however, this decrease was reversed by RDN (P < 0.01) (Fig. 7b).

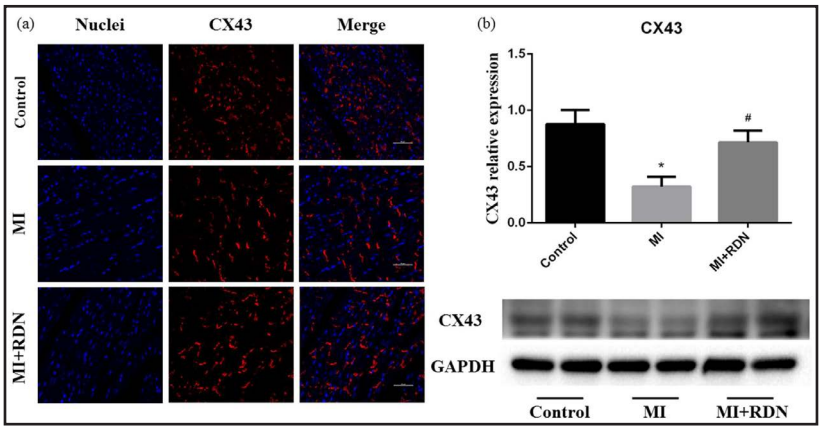

Fig. 7. Distribution and expression of Cx43 in the border-zone of the infarcted area from the control, MI, and MI+RDN groups.The distribution of Cx43 was disrupted in the border-zone of the infarcted area and the chaos of Cx43 was attenuated by RDN (a). Quantitative analysis and representative protein expression of Cx43 in heart by Western blot (b). (n = 8, 9, 8 in Control, MI and MI+RDN group, Data were mean ± SD. *P<0.05 vs. Control group; # P<0.05 vs. MI group).

Discussion

The main findings of this study are as follows: (1) RDN attenuates VA inducibility in ischemic cardiomyopathy; (2) RDN significantly reduces myocardial fibrosis in ischemic cardiomyopathy; (3) RDN abolishes the abnormal distribution and quantity of Cx43; and (4) RDN has protective effects against cardiac dysfunction in MI rats.

Malignant VAs accompanied with severe hemodynamic disturbances are the major cause of sudden cardiac death after MI [1, 14]. Medical treatment and an implantable cardioverter defibrillator (ICD) are the most commonly used therapeutic approaches for VAs. However, the development of anti-arrhythmic drugs alone is not sufficient, while the implantation of a cardioverter defibrillator is very expensive and has several side effects [15]. Thus, new techniques should be explored for the treatment of VAs.

Cardiac dysfunction is one of the predictors of VAs in ischemic cardiomyopathy [16]. In the present study, left ventricular dilation, compensatory myocardial hypertrophy, and poor cardiac function occurred in rats that suffered a coronary occlusion at 2 weeks after MI. However, impaired heart function was partially restored through the RDN procedure. In addition, the plasma levels of BNP, which is mainly released from the ventricles and is associated with a higher mortality in heart failure [17], were significantly decreased in MI rats with RDN compared with the MI group. These observations were similar to the findings of Hu et al. [18, 19].

Previous studies have investigated the influences of RDN on VAs in ischemic heart disease. Huang et al. demonstrated that RDN can decrease the incidence of VAs in an experimental model of acute myocardial infarction in dogs [20]. Similarly, Linz et al. reported that RDN suppressed VAs during acute ischemic events in pigs [6]. However, only a few studies have focused on the role of RDN against VAs in a model of MI-induced ischemic cardiomyopathy. Nicholas et al. recently showed that RDN can reduce spontaneous VAs after MI, and this phenomenon may be associated with the downregulation of cardiac sympathetic activity [7]. In the present study, RDN indeed decreased the occurrence of VAs in ischemic heart disease, which was consistent with the findings of Jacksonet al. [7].

Fibrosis is a common pathway leading to organ injury and failure [21]. In response to variable environment stimuli, including myocardial infarction, structural remodeling occurs in the heart, with a massive deposition of extracellular matrix. These pathological changes may increase the risks of heart failure and malignant arrhythmias [22, 23]. In fact, a study showed that a 3% increase in extracellular volume fraction contributes to a 50% increase in cardiovascular events [24]. In our study, the protein expression levels of TGF-β1 and α-SMA, which are common fibrosis-related factors, were significantly downregulated, and

the plasma levels of PINP and PIIINP, known as specific biomarkers involved in collagen type I and III synthesis [25], were decreased in the MI+RDN group compared with the MI group. Thus, the RDN procedure indeed reduced myocardial fibrosis in ischemic hearts and could reduce the frequency of VAs.

Numerous gap junctions located between cardiomyocytes facilitate conduction through normal ventricular muscle in a continuous process [26]. However, there are fewer and smaller gap junctions between myocytes located near infarcts [27] Thus, conduction velocity is reduced. Actually, conditions that decrease conduction velocity also promote reentry, while reentry plays a vital role in the formation of VAs in infarcted hearts [26]. In the present study, the abnormal distribution and quantity of Cx43 were attenuated by the RDN procedure, which may help to improve conduction velocity and reduce reentry. Therefore, the effects of RDN on antiarrhythmic were probably related to the preservation of Cx43 in infarcted hearts.

Several limitations of this study should be noted. First, the major etiology of clinical MI is coronary atherosclerosis, while we induced MI by ligating the left anterior descending coronary artery. As a result, the pathophysiological state of the experimental ischemic cardiomyopathy model may be different from the actual state. Second, epicardial monophasic action potentials were not recorded during PES, so we could not calculate action potential duration at 90% repolarization or the dispersion of action potential duration, which are both increased in post-infarcted hearts [12].

Conclusion

RDN reduced myocardial fibrosis and suppressed VAs in a rat model of ischemic cardiomyopathy.

Acknowledgements

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Disclosure Statement

The authors declare that no conflict of interests exists.

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