

Original Article

Renal sympathetic denervation raises blood pressure and attenuates ventricular remodeling in rats with myocardial infarction

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Abstract: Renal denervation (RDN) is reported to suppress the sympathetic nervous system and renin-angiotensin system, thereby, lowering blood pressure in patients with resistant hypertension. However, the effect and safety of RDN on ventricular remodeling following myocardial infarction (MI), especially with hypotension, is unknown. Forty rats were randomized into: MI, MI+RDN, Sham and RDN groups to examine the impact of RDN on blood pressure and ventricular remodeling in MI rats. **Results:** Compared with the MI group, MI+RDN showed lower levels of plasma norepinephrine, angiotensin II, cardiac angiotensin II, and angiotensin II type 1 receptor ($P<0.05$). On days 21 and 28, there was a distinct increase of SBP in the MI+RDN group, compared with the MI group ($P<0.05$). Compared with the MI group on days 14, 21 and 28, a significant increase of DBP in the MI+RDN group was observed ($P<0.05$). RDN ameliorated decreases of ejection fraction and fractional shortening, as well as increases in left ventricular internal dimensions in MI rats ($P<0.05$). RDN also caused decreases of collagen volume fraction in MI rats and inhibited the deposition of Collagens I and III, as well as the expression of transforming growth factor- β 1 (TGF- β 1) ($P<0.05$). In conclusions, RDN raises blood pressure and improves post-infarction ventricular remodeling in MI rats, probably due to the suppression of TGF- β 1 expression and collagen deposition via inhibition of SNS and RAS. RDN is a novel anti-fibrotic method for post-infarction remodeling and may be used in other cardiovascular diseases with similar pathological processes.

Keywords: Hypotension, myocardial infarction, remodeling, renal denervation, sympathetic nerve

Introduction

Myocardial infarction (MI) due to coronary artery occlusion is one of the leading causes of death in the world [1]. Myocardial injury following MI activates the sympathetic nervous system (SNS) and renin-angiotensin system (RAS) to maintain hemodynamic stability, which also results in the post-infarction ventricular remodeling and functional deterioration. The medications designed to block neurohormonal activation are common therapies for reversing post-infarction remodeling in clinical practice, including angiotensin converting enzyme inhibitors (ACEIs), angiotensin II type 1 receptor blockers (ARBs), β -adrenergic blockers, and aldosterone

receptor blockers [2]. Unfortunately, many patients cannot tolerate these medications for their side effects, drug-to-drug interactions, and long-term economic burdens [3].

Previous studies have described catheter-based renal denervation (RDN) as an effective treatment for patients with resistant hypertension via the suppression of SNS and RAS [4-7]. Moreover, a growing number of studies have demonstrated the application of RDN treatment in animal models with cardiovascular diseases, such as heart failure following MI, cardiac dysfunction with pressure overload, and pulmonary vascular remodeling after pulmonary artery hypertension [8-11]. Among them, Hu et

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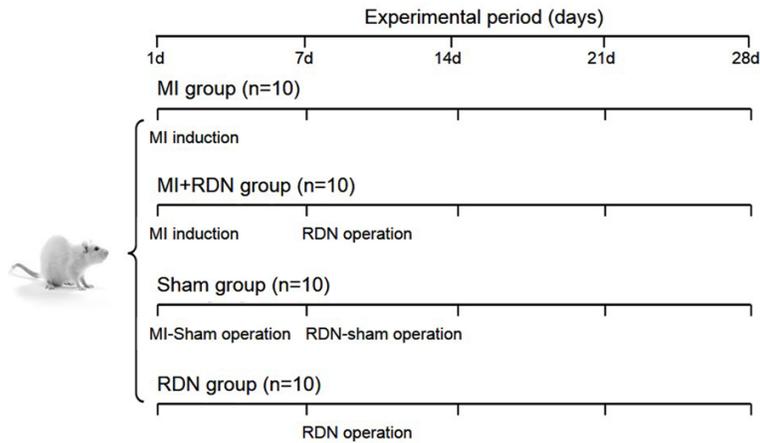


Figure 1. The experimental design of the four groups. To evaluate the effect of RDN operation on MI rats, 40 rats were randomly assigned to four groups: MI group, MI+RDN group, Sham group, and RDN group (n = 10 in each group). The experimental design scheme shows the MI induction time (day 1) and RDN operation time (day 7). Abbreviations: MI, myocardial infarction; RDN, renal denervation.

al. reported RDN was effective in rats with heart failure via promoting a robust diuresis and natriuresis [9]. Doltra *et al.* found RDN significantly decreased the left ventricular mass due to the reduction of collagen content in patients with resistant hypertension [12]. Particularly, post-infarction remodeling is characterized by excess deposition of Collagen I and III in the border area adjacent to the infarcted myocardium [13]. High expression of transforming growth factor (TGF)- β 1 decreases the expression of collagenase, and enhances the synthesis and deposition of Collagen I and III [14, 15]. The effect of RDN in halting collagen deposition during post-infarction ventricular remodeling is still unclear. The impact of RDN on blood pressure in MI patients is also unknown.

To explore the effect and safety of RDN on post-infarction ventricular remodeling, we created experimental MI rat models and denervated their bilateral renal nerves one week after MI induction. Electrical stimulations and molecular changes of SNS and RAS were measured to evaluate the denervation effect of the RDN operation. Dynamic changes of blood pressure and post-infarction remodeling were monitored to test the effect and safety of RDN on MI rat models. Expression changes of Collagen I, III and TGF- β 1 were further measured to explore the underlying mechanisms of RDN on post-infarction remodeling.

Material and methods

Ethics statement

The use and care of animals were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals. All procedures were approved by the Committee on the Ethics of Animal Experiments of the Second Military Medical University (Permit Number: 2012-0003). All efforts were made to reduce the number of animals used and minimize their suffering through the use of anesthesia, analgesia and monitoring.

Experimental animals

A total of 40 male Wistar rats (180-220 g) were obtained from the experimental animal center of the Second Military Medical University (Shanghai, China). Rats were housed at constant room temperature (22-25°C), humidity (55-60%) and light cycle (12:12 h light-dark), with free access to tap water and standard rat chow.

The rats were randomly assigned to four groups: MI group (rats with MI induction, n = 10), MI+RDN group (rats with RDN operation one week after MI induction, n = 10), RDN group (rats with RDN operation, n = 10), Sham group (rats without MI induction or RDN operation, n = 10) (**Figure 1**). Rats in the Sham group were sham-operated using similar procedures without coronary artery ligation or renal denervation.

Coronary artery ligation

To prepare the MI model, coronary artery ligation was carried out on MI group rats on day 1 of the experimental period. Under ether anesthesia (5 ml/l air), the heart was exteriorized via the fourth intercostal space without intubation. The left coronary artery was ligated within 3 mm from its origin quickly and exactly. After ligation, the heart was placed back into the intrathoracic space followed by manual evacuation of air immediately. The rat was then returned to its home cage and allowed to breathe room air. A successful MI model was

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certified by electrocardiogram showing ST segment elevation. In the Sham group, sham-operation was performed on rats using similar procedures, but without coronary artery ligation.

Bilateral renal denervation

To evaluate the impact of RDN on the MI rat model, bilateral renal denervation operation was carried out on MI+RDN and RDN group rats on day 7 of the experimental period. Rats were anesthetized with 10% chloral hydrate (3-5 ml/kg, i.p.). After a midline abdomen incision, and exposure of subcutaneous tissues and kidneys, renal denervation was performed bilaterally by stripping the adventitia of renal artery and vein. All visible renal nerve bundles were dissected and 10% phenol in 95% ethanol was used to further ablate the tissues surrounding the vessels.

The success of RDN operation was primarily evaluated by comparing the electrical stimulation of proximal renal vessels for 10-30 seconds before and after the operation. Successful RDN operation was identified as no changes in blood pressure, heart rate or color of the kidney observed after bilateral denervation. After the RDN operation, the wound was closed and rats were allowed to wake spontaneously. In Sham group, sham-operation was performed in rats using similar procedures but without renal denervation.

Blood pressure monitoring and echocardiographic measurement

To test the effect and safety of RDN on MI rats, blood pressure was recorded by a computerized tail-cuff system (ALC-NIBP, Shanghai Alcott Biotech, China) on days 1, 7, 14, 21 and 28. On day 28, echocardiography was performed with a high-resolution small animal echocardiographic system, equipped with a cardiac probe (RMV716, VisualSonics, Canada). Each rat was anesthetized with ketamine hydrochloride (50 mg/kg, i.p.). Left ventricular internal dimensions at end systole (LVIDS) and end diastole (LVIDD), ejection fraction (EF) and fractional shortening (FS) were measured from at least three consecutive cardiac cycles. All measurements were carried out by two technicians who were unaware of the identities of experimental groups.

ELISA and western blot analysis

To further confirm the success of the RDN operation, molecular changes of SNS and RAS were measured by enzyme-linked immunosorbent assay (ELISA) and western blot, as reflected by plasma norepinephrine (NE), angiotensin II (Ang II), and cardiac Ang II and Ang II type 1 receptor (AT1R).

Rats were sacrificed by cervical dislocation after echocardiographic data collection and blood was obtained from the heart. Plasma NE and Ang II were measured by ELISA (ELISA Kit, Nanjing Jiancheng Bioengineering Institute, China). The heart was then quickly removed and the left ventricle was separated for preparation of tissue samples. The method for the determination of infarct size and border area was reported previously [16]. Parts of myocardium were stored at -80°C for the following molecular analysis. Expression of Ang II in the myocardium was measured using ELISA Kit. Expression of AT1R in the myocardium was measured by western blot. The membranes were blocked with 5% non-fat dry milk and incubated overnight with primary antibody (AT1R, 1:1000; GAPDH, 1:10000; Abcam, UK) at 4°C overnight and subsequently with secondary antibody at 37°C for 1 hour. The intensity of each band was quantified using ImageJ analysis software (National Institutes of Health, USA). Each value was normalized to GAPDH in individual samples.

Histological evaluation

The rest left ventricle was fixed in 4% paraformaldehyde and embedded in paraffin for histological change analysis. Masson's trichrome staining was used to identify collagen deposits in the border area. Sections were imaged at 100× magnification using bright-field microscopy (AxioCam HRc, Zeiss, Germany). Collagen volume fraction (CVF) in the ventricle was calculated to assess the quantity of myocardial fibrosis. CVF was determined as the ratio of collagen surface area stained blue with respect to myocardial surface area. All quantitative evaluations were carried out by ImagePro Plus software (Version 6.0, Media Cybernetics, USA).

Immunohistochemical staining and quantitative RT-PCR (qRT-PCR) analysis

To explore the mechanisms of RDN on MI rats, the protein expression of Collagen I, Collagen III

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Table 1. Primers used in quantitative real-time PCR analysis

Collagen I	Forward primer	TGCTTGAAGACCTATGTGGGTA
	Reverse primer	AAAGGCAGCATTGGGGTAT
Collagen III	Forward primer	GAGGAATGGGTGGCTATCCT
	Reverse primer	GGTATCCAGGAGAACCAGGAG
TGF-β1	Forward primer	CCTGGAAAGGGCTCAACAC
	Reverse primer	CAGTTCTTCTGTGGAGCTGA
GAPDH	Forward primer	AACGACCCCTTCATTGACCTC
	Reverse primer	CCTTGACTGTGCCGTTGAAT

Table 2. General data of rats in the four groups

	MI	MI+RDN	Sham	RDN
Mortality (%)	20.0	30.0	0	0
Number	8	7	10	10
BW (g)	321.45±9.91 ^Δ	315.60±8.11 ^Δ	354.30±9.20	341.10±10.83
LV/BW (mg/g)	2.06±0.12	2.14±0.10	1.98±0.13	1.96±0.11
Infarct size (%)	39.09±5.39	37.00±4.83	0	0

BW, body weight; LV/BW, ratio of left ventricle to body weight. * $P < 0.05$ vs MI group, ^Δ $P < 0.05$ vs Sham group.

and TGF-β1 in the border area were measured using immunohistochemical staining. Tissue sections were deparaffinized and rehydrated through graded alcohol for immunohistochemical staining. Sections were incubated in 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity, and then received 5% bovine serum albumin for 20 minutes to block non-specific binding. Following washing in phosphate buffered saline, sections were treated with primary antibody (Collagen I, 1:100; Collagen III, 1:200; TGF-β1, 1:400; Abcam, UK) at 4°C overnight and subsequently with secondary antibody at 37°C for 1 hour. Visualization was done with diaminobenzidine and sections were observed (brown color) at 200× magnification under light microscope (Axio Scope, Zeiss, Germany).

The mRNA expression of Collagen I, Collagen III and TGF-β1 were also measured using qRT-PCR analysis. Total RNA samples in the border area were isolated using TRIzol reagent according to the manufacturer's protocol (Invitrogen, USA). About 500 ng of total RNA was used to generate cDNA by using oligo-dT₁₆ for mRNAs. qRT-PCR was performed with SYBR Green Mix kit (TAKARA, China). The amount of target gene (Collagen I, Collagen III and TGF-β1) was normalized to the amount of endogenous control (GAPDH) and the results were given by $2^{-\Delta\Delta Ct}$ relative to the control samples. Primers used in

the amplification reaction were described in **Table 1** (shown 5'-3').

Statistical analysis

All continuous variables are expressed as mean values and standard deviations, and discrete variables are presented as percentages. Differences among multiple groups were determined by a one-way ANOVA, and in the case of a significant difference, further analysis was undertaken with a Post hoc Scheffe test. A value of $P < 0.05$ was considered statistically significant. Data were analyzed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

Results

General data

The baseline characteristics are summarized in **Table 2**. During 28 days of observation, 2 rats died of ventricular fibrillation in the MI group. In the MI+RDN group, 2 rats died of ventricular fibrillation and the other one died of serious hemorrhage. There were no significant differences in mortality and infarct area ratio between the MI group and MI+RDN group ($P > 0.05$). No rats in the Sham group or RDN group died during the experiment.

RDN attenuated the activation of SNS and RAS in MI rats

To assess the effect of RDN on the activation of SNS and RAS after MI, plasma levels of NE and Ang II, and cardiac levels of Ang II and AT1R were measured. As shown in **Figure 2A-C**, compared with the Sham group, there was a significant increase in the levels of plasma NE, Ang II and cardiac Ang II ($P < 0.05$), consistent with previous reports [17]. Compared with the MI group, MI+RDN group showed lower levels of plasma NE, Ang II and cardiac Ang II ($P < 0.05$). As shown in **Figure 2D, 2E**, RDN also caused a lower expression of cardiac AT1R in the border area of MI rats.

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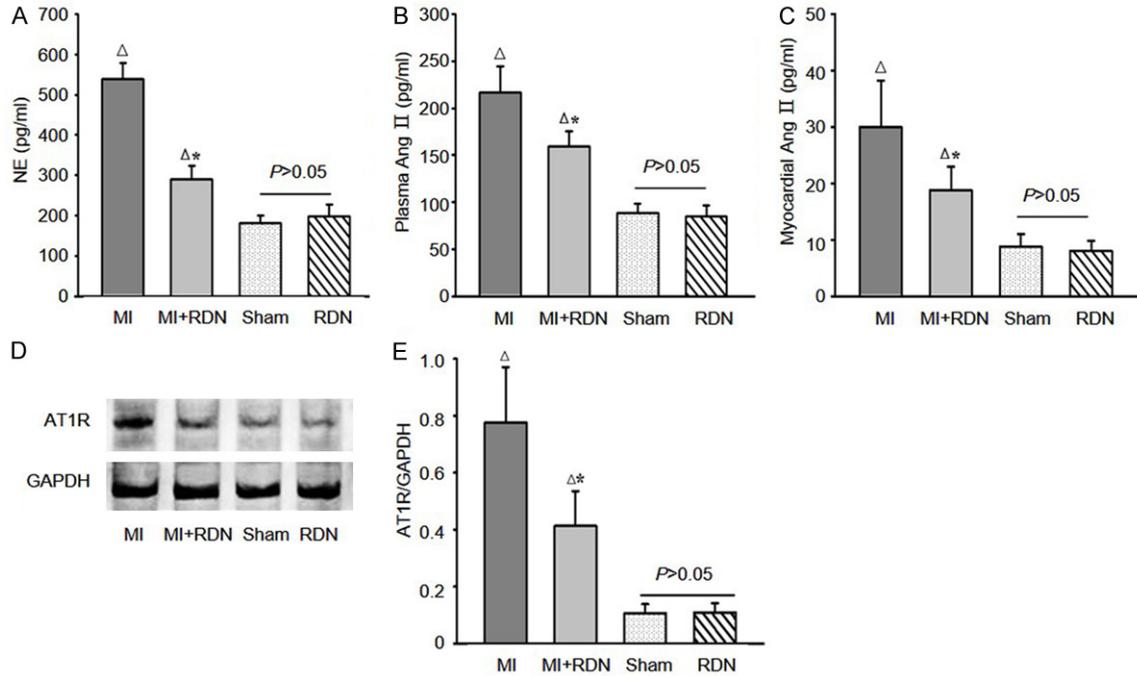


Figure 2. RDN attenuated the activation of SNS and RAS in MI rats. Compared with the MI group, MI+RDN group showed lower levels of plasma NE (A), plasma Ang II (B), and cardiac Ang II (C) ($P<0.05$). RDN also caused a lower expression of AT1R in the border area of MI rats (D, E). Abbreviations: Ang II, angiotensin II; AT1R, Ang II type 1 receptor; MI, myocardial infarction; NE, norepinephrine; RAS, renin-angiotensin system; RDN, renal denervation; SNS, sympathetic nerve system. * $P<0.05$ vs MI group, $^{\Delta}P<0.05$ vs Sham group.

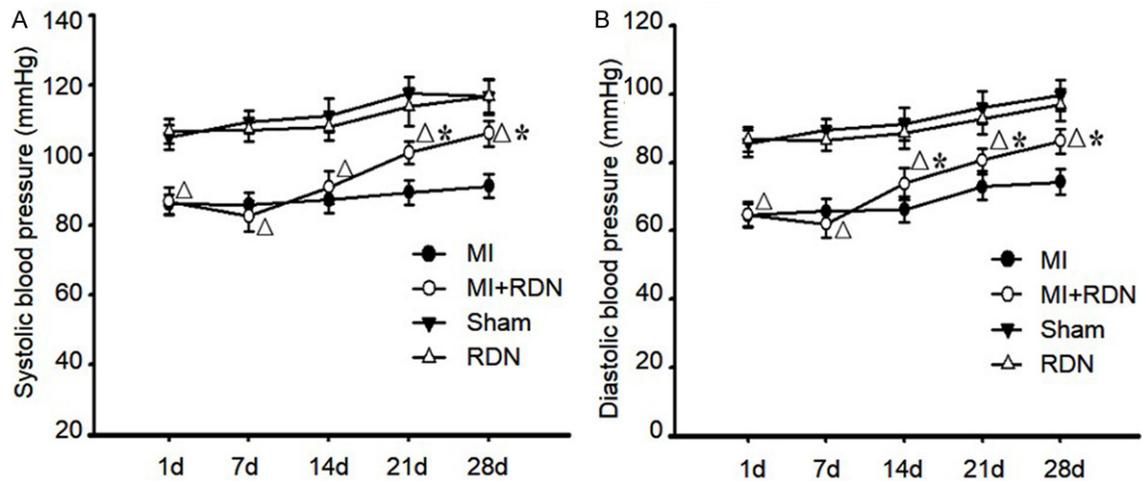


Figure 3. RDN raised blood pressure in MI rats. A. Compared with the MI group, there was a significant increase of SBP in the MI+RDN group on day 21 and day 28 of the experimental period ($P<0.05$). B. Compared with the MI group, there was a significant increase of DBP in the MI+RDN group on day 14, day 21 and day 28 of the experimental period ($P<0.05$). Abbreviations: DBP: diastolic blood pressure; MI, myocardial infarction; RDN, renal denervation; SBP, systolic blood pressure. * $P<0.05$ vs MI group, $^{\Delta}P<0.05$ vs Sham group.

RDN raised blood pressure in MI rats

To test the safety of RDN on MI rats, dynamic changes of blood pressure were monitored in

each group. As shown in **Figure 3A, 3B**, both systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the MI+RDN group and MI group were significantly lower than the Sham

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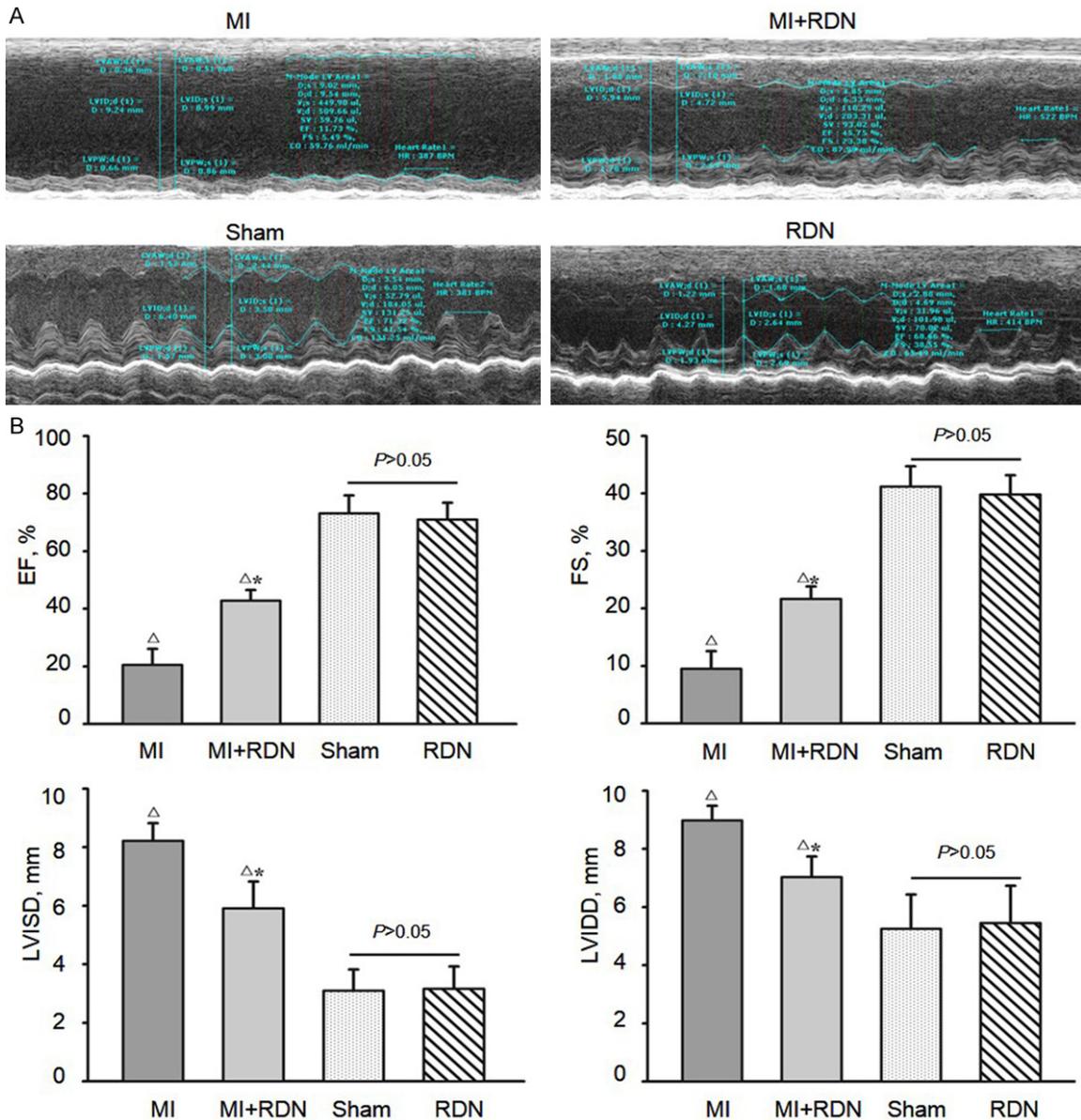


Figure 4. RDN improved ventricular remodeling in MI rats. A. Representative pictures of M-mode echocardiography of the 4 groups. Compared with sham-operated rats, MI rats displayed a larger left ventricular cavity, flatter motion curve, and worse contractile function, which were improved by RDN operation in the MI+RDN group. No significant changes were observed between the Sham group and RDN group. B. Analysis of echocardiographic measurements among the 4 groups. Compared with the Sham group, MI caused a progressive impairment of cardiac systolic and diastolic function, as reflected by the decreases of EF and FS and increases of LVISD and LVIDD ($P < 0.05$). Compared with the MI group, RDN ameliorated the decreases of EF and FS, and increases of LVISD and LVIDD, in the MI+RDN group ($P < 0.05$). No significant differences were detected in the EF, FS, LVISD, and LVIDD between the Sham group and RDN group ($P > 0.05$). Abbreviations: EF, ejection fraction; FS, fractional shortening; LVISD, left ventricular internal dimensions at end systole; LVIDD, left ventricular internal dimensions at end diastole; MI, myocardial infarction; RDN, renal denervation. * $P < 0.05$ vs MI group, $^{\Delta}P < 0.05$ vs Sham group.

and RDN groups on day 1, after MI induction ($P < 0.05$). No significant difference of SBP and DBP between the MI+RDN group and MI group were observed on day 1 ($P > 0.05$). On day 7, compared with the MI group, there was a slight

but not significant decrease of SBP and DBP in MI+RDN group ($P > 0.05$). On day 21 and day 28, there was a distinct increase of SBP in the MI+RDN group when compared with the MI group ($P < 0.05$). On days 14, 21, and 28, com-

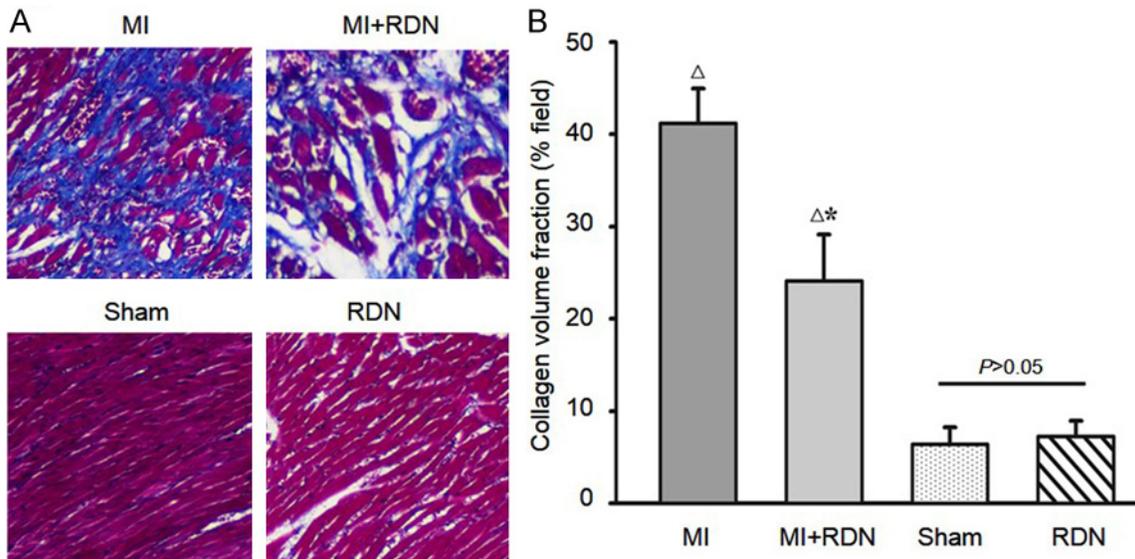


Figure 5. RDN attenuated the CVF in MI rats. A. Representative pictures of the Masson's trichrome-stained myocardial tissues. Compared with the MI group, MI+RDN group showed decreased marked collagen deposition (blue color, magnification $\times 200$). B. Quantitative analysis of CVF among the 4 groups. RDN caused a substantial decrease of the CVF in the MI+RDN group compared with the MI group ($P < 0.05$). No significant changes were observed in the CVF between the Sham group and RDN group ($P > 0.05$). Abbreviations: CVF, collagen volume fraction; MI, myocardial infarction; RDN, renal denervation. $*P < 0.05$ vs MI group, $^{\Delta}P < 0.05$ vs Sham group.

pared with the MI group, a significant increase of DBP in the MI+RDN group was observed ($P < 0.05$).

However, we found RDN could not restore blood pressure back to normal in the MI+RDN group when compared with the Sham group ($P > 0.05$). Moreover, no significant difference in blood pressure between the Sham and RDN groups were observed during the experiment ($P > 0.05$).

RDN improved ventricular remodeling in MI rats

To evaluate the effect of RDN on ventricular remodeling, echocardiography was performed to observe cardiac structural and functional changes. As shown in **Figure 4A**, M-mode echocardiographic images demonstrated MI rats had a larger left ventricular cavity, flatter motion curve, and worse contractile function as compared with sham-operated rats. Three weeks after RDN operation in MI rats, the cardiac function in the MI+RDN group improved as evidenced by increased left ventricular cavity, and restored motion curve and contractile function.

As shown in **Figure 4B**, compared with the Sham group, MI caused a progressive impairment of echocardiographic measurements as

reflected by the decreases in the EF and FS, and increases in the LVIDS and LVIDD ($P < 0.05$). Compared with the MI group, RDN ameliorated the decreases of EF and FS, and the increases of LVIDS and LVIDD ($P < 0.05$). No significant changes were observed in the echocardiographic measurements between the Sham group and RDN group ($P > 0.05$).

RDN attenuated the CVF in MI rats

To observe the effect of RDN on collagen deposition after MI, Masson's trichrome staining was used to identify collagen deposition, and CVF was applied to quantify fibrotic area. As shown in **Figure 5A**, red color indicates muscle fibers, black indicates nuclei, and blue indicates interstitial collagen. Compared with the MI group, MI+RDN group showed decreased marked collagen deposition. As shown in **Figure 5B**, RDN caused a substantial decrease of the CVF in the MI+RDN group compared with the MI group ($P < 0.05$). No significant changes were observed in the CVF between the Sham group and RDN group ($P > 0.05$).

RDN inhibited collagen deposition and TGF- $\beta 1$ expression in MI rats

To evaluate the effect of RDN on cardiac collagen deposition, we used immunohistochemical

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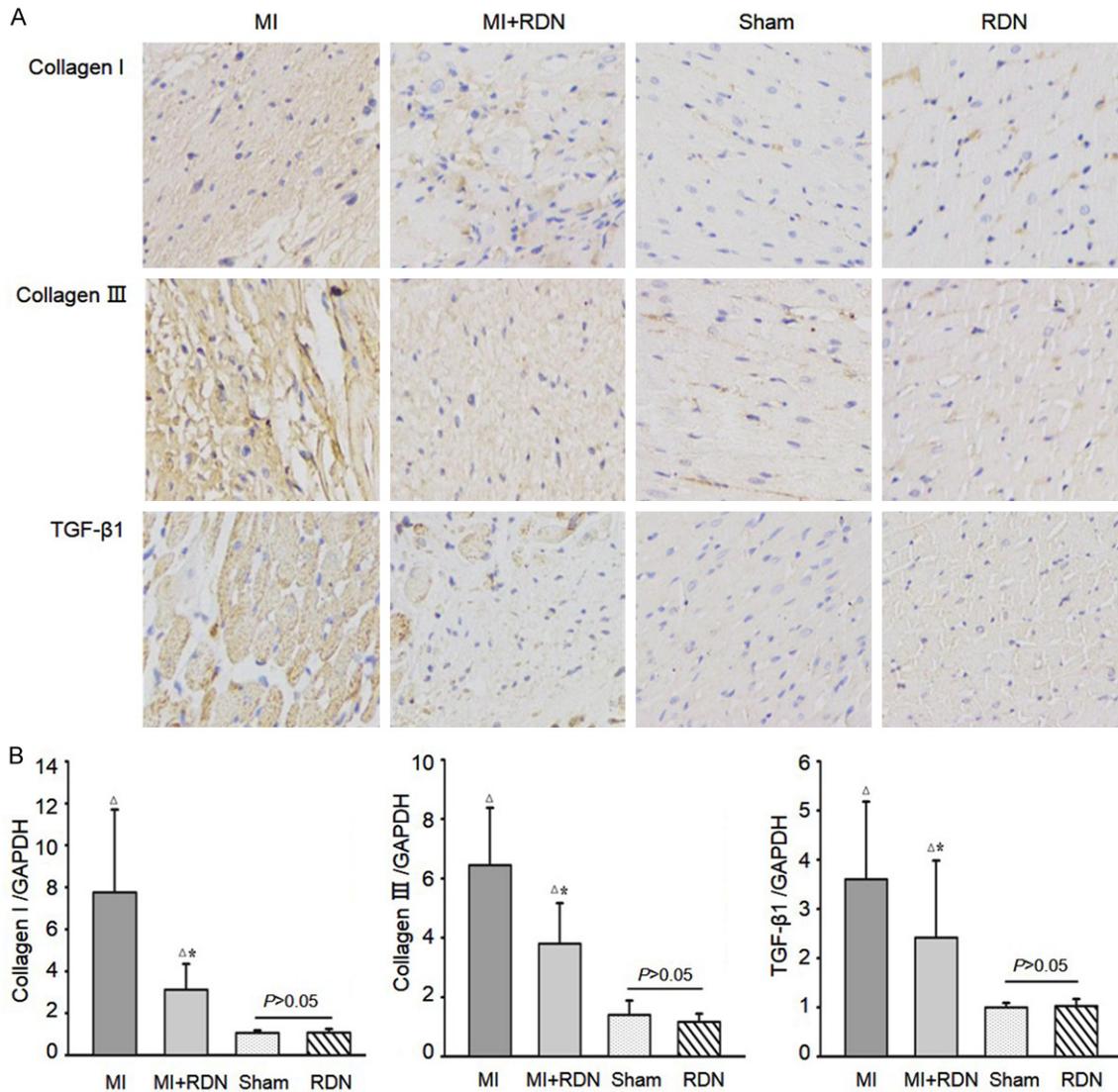


Figure 6. RDN inhibited collagen deposition and TGF-β1 expression in MI rats. A. Representative pictures of immunohistochemical-stained myocardial tissues. Compared with the MI group, MI+RDN group showed lower protein expression of Collagen I, Collagen III, and TGF-β1 (brown color, magnification $\times 200$). B. qRT-PCR analysis of mRNA levels of Collagen I, Collagen III, and TGF-β1 among the 4 groups. RDN caused a significant decrease of Collagen I, Collagen III, and TGF-β1 in the MI+RDN group compared with the MI group ($P < 0.05$). No significant changes were observed in the collagen deposition and TGF-β1 expression between the Sham group and RDN group ($P > 0.05$). Abbreviations: MI, myocardial infarction; RDN, renal denervation; TGF-β1, transforming growth factor-β1. * $P < 0.05$ vs MI group, $\Delta P < 0.05$ vs Sham group.

staining to observe the protein expression of Collagen I and Collagen III in the border area of MI rats. As shown in **Figure 6A**, RDN inhibited the protein expression of Collagen I and Collagen III in the MI rats. Since TGF-β1 is an important profibrotic cytokine during cardiac remodeling, we measured the protein expression of TGF-β1 and found RDN operation could counteract the high expression of TGF-β1 in MI rats.

We further analyzed the mRNA levels of Collagen I, Collagen III, and TGF-β1 by qRT-PCR. As shown in **Figure 6B**, RDN significantly attenuated the mRNA levels of Collagen I and Collagen III in MI rats ($P < 0.05$). RDN also inhibited the mRNA level of TGF-β1 in MI rats ($P < 0.05$). As expected, there was no significant difference in the protein and mRNA levels of Collagen I, Collagen III, or TGF-β1 between the Sham group and RDN group ($P > 0.05$).

Discussion

In the present study, we evaluated the effect and safety of RDN on post-infarction remodeling in experimental MI rats. The success of RDN operation was confirmed by electrical stimulations and molecular changes of SNS and RAS. We found RDN gradually raised blood pressure of MI rats, although not back to normal levels. RDN reduced the deposition of Collagen I and Collagen III, and inhibited the expression of TGF- β 1 in the border area of MI rats, which was associated with an overall beneficial effect on ventricular remodeling and cardiac function. The present findings suggest that RDN is a potentially safe and effective alternative for halting cardiac fibrosis and attenuating post-infarction ventricular remodeling in MI patients.

It has been well known that the activation of SNS and RAS play an important role in the development of hypertension and ventricular remodeling. The earlier Simplicity Hypertension-1 (HTN-1) and HTN-2 trials demonstrated the catheter-based RDN as an alternative strategy for the treatment of resistant hypertension via the inhibition of SNS and RAS [4-7]. However, recently published HTN-3 trial resulted in a negative outcome and cast a pall over the renal denervation [18]. Criticism is raised over the inexperience of operators and likelihood that renal denervation was actually achieved, since it was not evaluated by the operators in HTN-3 trial. Thus increased efforts are necessary to validate the success of denervation operation.

In the present study, we preliminarily evaluated the success of RDN operation via electrical stimulations, as no changes in blood pressure, heart rate, or color of the kidney were observed after bilateral denervation. We then tested the molecular levels of SNS and RAS. Currently, the activity of SNS is difficult to evaluate. Circulating plasma NE measurement is recognized as a crude guide of SNS because it depends on the rate of immediate NE reuptake as well as NE clearance from circulation [19]. Ang II is known as the principle effector of RAS. The effect of Ang II on ventricular remodeling is mediated via its binding of AT1R in the border area of local myocardium [20, 21]. In our study, we found RDN decreased the release of NE and

Ang II in circulating plasma and reduced the expression of Ang II and AT1R in local myocardium of MI rats. Taken together, our study confirmed RDN was effectively performed in individual rats, via electrical stimulations and molecular measurements of SNS and RAS.

RDN is reported to be effective for resistant hypertension but its impact on blood pressure in MI patients is unclear. We investigated the effect of RDN on blood pressure in MI rats and found RDN did not lower blood pressure but caused a significant increase of SBP and DBP. In addition to raising blood pressure, RDN led to decreased LVDS and LVDD and increased EF and FS in MI rats. We speculated the beneficial effect of RDN on blood pressure of MI rats was the consequence of RDN improving the cardiac function and cardiac output on post-infarction remodeling. Our echocardiographic results were consistent with previous findings, which reported the effect of RDN on left ventricular remodeling was due to the increase of water and sodium excretion in MI rats [9, 22]. However, Watanabe *et al.* did not observe any changes in renal sodium excretion or daily sodium/water balance after RDN operation in hypertensive rats [23]. Till now, the impact of RDN on water and sodium excretion is still unclear.

Collagen deposition is known to play an important role in the generation and development of cardiac remodeling [24, 25]. Collagen deposition changes were sufficient to cause cardiac fibrosis in post-infarction remodeling. Collagen I represents about 80-85% of the total cardiac collagen content in the normal myocardium and Collagen III accounts for 10-15% of myocardial collagen content [26, 27]. Collagen I is the major determinant of myocardial diastolic stiffness at sites of border area [28]. Down-regulation of collagen deposition during healing phases is of great benefit to cardiac function after MI [13, 29]. In our study, we found RDN one week after MI attenuated the increase of CVF and reduced the deposition of Collagen I and Collagen III in the border area of MI rats. The changes of Collagen I and Collagen III were in line with the improvement of ventricular remodeling, suggesting that the beneficial effect of RDN on MI rats was the consequence of affecting collagen deposition and cardiac fibrosis.

TGF- β 1, as a profibrotic cytokine, is highly expressed in the border area of infarct region and interacts with local RAS, thereby playing a vital role in the collagen deposition and ventricular remodeling [30, 31]. We investigated the levels of TGF- β 1 and found RDN attenuated the high expression of TGF- β 1 in the border area of MI rats. Rosenkranz *et al.* reported that Ang II induced collagen synthesis via the upregulation of TGF- β 1 in the local myocardium [32]. Following the treatment of Ang II receptor antagonist losartan, the expression of TGF- β 1 and Collagen I were both reduced significantly in the border area [33]. Zheng *et al.* reported RDN might affect matrix metalloproteinases via TGF- β 1 signaling in myocardial remodeling [34]. In our study, we found following RDN operation, the suppression of TGF- β 1 expression and collagen deposition was consistent with the inhibition of SNS and RAS in MI rats. Thus we propose the effect of RDN on ventricular remodeling is attributed to the suppression of TGF- β 1 expression and collagen deposition, through its inhibition of SNS and RAS.

Some limitations should be considered in interpreting the present results. We performed RDN treatment one week after MI induction to evaluate the impact of RDN on post-infarction remodeling. Kline *et al.* reported renal nerve regeneration occurred after 8 weeks following RDN operation [35]. We observed RDN was persistently effective within 4 weeks after RDN operation. Our results suggest RDN is safe and effective at initial stages of post-infarction remodeling. We are also very interested in observing the effect and safety of RDN treatment during a different time course of ventricular remodeling.

In summary, our findings demonstrated that RDN may inhibit the activity of SNS and RAS, and then reduce TGF- β 1 expression and collagen deposition in the border area of MI rats. These histological and molecular changes were associated with the improvement of blood pressure and ventricular remodeling in MI rats. Our data is preliminary but RDN may be a safe and effective alternative for the treatment of post-infarction patients especially with hypotension status. Although the precise mechanism of RDN treatment is not certain, we confirm the therapeutic potential of RDN specially targeting the collagen deposition and TGF- β 1 pathway following MI. Furthermore, cardiac fibrosis is a

pathological feature common to numerous forms of heart diseases. The present study identified the RDN treatment as a novel anti-fibrotic method, which might be used in the cardiovascular diseases with similar pathological processes in the future.

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Disclosure of conflict of interest

None.

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References

- [1] Schwartz Longacre L, Kloner RA, Arai AE, Baines CP, Bolli R, Braunwald E, Downey J, Gibbons RJ, Gottlieb RA, Heusch G, Jennings RB, Lefer DJ, Mentzer RM, Murphy E, Ovize M, Ping P, Przyklenk K, Sack MN, Vander Heide RS, Vinten-Johansen J, Yellon DM; National Heart, Lung, and Blood Institute, National Institutes of Health. New horizons in cardioprotection: recommendations from the 2010 National Heart, Lung, and Blood Institute Workshop. *Circulation* 2011; 124: 1172-1179.
- [2] Khattar RS. Effects of ACE-inhibitors and beta-blockers on left ventricular remodeling in chronic heart failure. *Minerva Cardioangiologica* 2003; 51: 143-154.
- [3] Kirkpatrick JN and St John Sutton M. Assessment of ventricular remodeling in heart failure clinical trials. *Curr Heart Fail Rep* 2012; 9: 328-336.
- [4] Schlaich MP, Sobotka PA, Krum H, Whitbourn R, Walton A and Esler MD. Renal denervation as a therapeutic approach for hypertension: novel implications for an old concept. *Hypertension* 2009; 54: 1195-1201.
- [5] Krum H, Schlaich M, Whitbourn R, Sobotka PA, Sadowski J, Bartus K, Kapelak B, Walton A, Sievert H, Thambar S, Abraham WT and Esler M. Catheter-based renal sympathetic denervation

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- tion for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet* 2009; 373: 1275-1281.
- [6] Goliash G, Wolzt M, Hofer P, Delle-Karth G, Kreiner G, Neunteufel T, Maurer G and Binder T. [Percutaneous renal denervation in patients with resistant hypertension—first experiences in Austria]. *Wien Klin Wochenschr* 2010; 122: 723-726.
- [7] Mahfoud F, Ukena C, Schmieder RE, Cremers B, Rump LC, Vonend O, Weil J, Schmidt M, Hoppe UC, Zeller T, Bauer A, Ott C, Blessing E, Sobotka PA, Krum H, Schlaich M, Esler M and Bohm M. Ambulatory blood pressure changes after renal sympathetic denervation in patients with resistant hypertension. *Circulation* 2013; 128: 132-140.
- [8] Hu J, Li Y, Cheng W, Yang Z, Wang F, Lv P, Niu C, Hou Y, Yan Y and Ge J. A comparison of the efficacy of surgical renal denervation and pharmacologic therapies in post-myocardial infarction heart failure. *PLoS One* 2014; 9: e96996.
- [9] Hu J, Ji M, Niu C, Aini A, Zhou Q, Zhang L, Jiang T, Yan Y and Hou Y. Effects of renal sympathetic denervation on post-myocardial infarction cardiac remodeling in rats. *PLoS One* 2012; 7: e45986.
- [10] Li ZZ, Jiang H, Chen D, Liu Q, Geng J, Guo JQ, Sun RH, Zhu GQ and Shan QJ. Renal sympathetic denervation improves cardiac dysfunction in rats with chronic pressure overload. *Physiol Res* 2015; 64: 653-62.
- [11] Qingyan Z, Xuejun J, Yanhong T, Zixuan D, Xiaozhan W, Xule W, Zongwen G, Wei H, Shengbo Y and Congxin H. Beneficial Effects of Renal Denervation on Pulmonary Vascular Remodeling in Experimental Pulmonary Artery Hypertension. *Rev Esp Cardiol (Engl Ed)* 2015; 68: 562-70.
- [12] Doltra A, Messroghli D, Stawowy P, Hassel JH, Gebker R, Leppanen O, Grafe M, Schneeweis C, Schnackenburg B, Fleck E and Kelle S. Potential reduction of interstitial myocardial fibrosis with renal denervation. *J Am Heart Assoc* 2014; 3: e001353.
- [13] van den Borne SW, Diez J, Blankesteyn WM, Verjans J, Hofstra L and Narula J. Myocardial remodeling after infarction: the role of myofibroblasts. *Nat Rev Cardiol* 2010; 7: 30-37.
- [14] Border WA and Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994; 331: 1286-1292.
- [15] Eghbali M, Tomek R, Sukhatme VP, Woods C and Bhambi B. Differential effects of transforming growth factor-beta 1 and phorbol myristate acetate on cardiac fibroblasts. Regulation of fibrillar collagen mRNAs and expression of early transcription factors. *Circ Res* 1991; 69: 483-490.
- [16] Igawa A, Nozawa T, Yoshida N, Fujii N, Inoue M, Tazawa S, Asanoi H and Inoue H. Heterogeneous cardiac sympathetic innervation in heart failure after myocardial infarction of rats. *Am J Physiol Heart Circ Physiol* 2000; 278: H1134-1141.
- [17] Cohn JN, Ferrari R and Sharpe N. Cardiac remodeling—concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *J Am Coll Cardiol* 2000; 35: 569-582.
- [18] Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT, Leon MB, Liu M, Mauri L, Negoita M, Cohen SA, Oparil S, Rocha-Singh K, Townsend RR and Bakris GL. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med* 2014; 370: 1393-1401.
- [19] Esler M, Jennings G, Lambert G, Meredith I, Horne M and Eisenhofer G. Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiol Rev* 1990; 70: 963-985.
- [20] Weber KT, Sun Y and Campbell SE. Structural remodelling of the heart by fibrous tissue: role of circulating hormones and locally produced peptides. *Eur Heart J* 1995; 16 Suppl N: 12-18.
- [21] Dasgupta C and Zhang L. Angiotensin II receptors and drug discovery in cardiovascular disease. *Drug Discov Today* 2011; 16: 22-34.
- [22] Dai Z, Yu S, Zhao Q, Meng Y, He H, Tang Y, Wang X, Xiao J and Huang C. Renal sympathetic denervation suppresses ventricular substrate remodelling in a canine high-rate pacing model. *EuroIntervention* 2014; 10: 392-399.
- [23] Watanabe H, Iwanaga Y, Miyaji Y, Yamamoto H and Miyazaki S. Renal denervation mitigates cardiac remodeling and renal damage in Dahl rats: a comparison with beta-receptor blockade. *Hypertens Res* 2016; 39: 217-26.
- [24] Weber KT, Sun Y, Bhattacharya SK, Ahokas RA and Gerling IC. Myofibroblast-mediated mechanisms of pathological remodelling of the heart. *Nat Rev Cardiol* 2013; 10: 15-26.
- [25] Spinale FG, Gunasinghe H, Sprunger PD, Baskin JM and Bradham WC. Extracellular degradative pathways in myocardial remodeling and progression to heart failure. *J Card Fail* 2002; 8: S332-338.
- [26] Pelouch V, Dixon IM, Golfman L, Beamish RE and Dhalla NS. Role of extracellular matrix proteins in heart function. *Mol Cell Biochem* 1993; 129: 101-120.
- [27] Chapman D, Weber KT and Eghbali M. Regulation of fibrillar collagen types I and III and basement membrane type IV collagen gene expression in pressure overloaded rat myocardium. *Circ Res* 1990; 67: 787-794.

RDN on blood pressure and ventricular remodeling

- [28] Ertl G and Frantz S. Healing after myocardial infarction. *Cardiovasc Res* 2005; 66: 22-32.
- [29] Small EM, Thatcher JE, Sutherland LB, Kinoshita H, Gerard RD, Richardson JA, Dimaio JM, Sadek H, Kuwahara K and Olson EN. Myocardin-related transcription factor- α controls myofibroblast activation and fibrosis in response to myocardial infarction. *Circ Res* 2010; 107: 294-304.
- [30] Sun Y and Weber KT. Cardiac remodelling by fibrous tissue: role of local factors and circulating hormones. *Ann Med* 1998; 30 Suppl 1: 3-8.
- [31] Sutton MG and Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. *Circulation* 2000; 101: 2981-2988.
- [32] Rosenkranz S. TGF- β 1 and angiotensin networking in cardiac remodeling. *Cardiovasc Res* 2004; 63: 423-432.
- [33] Ju H, Zhao S, Jassal DS and Dixon IM. Effect of AT1 receptor blockade on cardiac collagen remodeling after myocardial infarction. *Cardiovasc Res* 1997; 35: 223-232.
- [34] Zheng XX, Li XY, Lyu YN, He YY, Wan WG, Zhu HL and Jiang XJ. Possible mechanism by which renal sympathetic denervation improves left ventricular remodeling after myocardial infarction. *Exp Physiol* 2016; 101: 260-271.
- [35] Kline RL and Mercer PF. Functional reinnervation and development of supersensitivity to NE after renal denervation in rats. *Am J Physiol* 1980; 238: R353-358.