



## Effects of renal sympathetic denervation on cardiac systolic function after myocardial infarction in rats

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### Abstract

This study investigated the therapeutic effects of renal denervation on cardiac systolic function after myocardial infarction (MI) in rats and the mechanism involved. Fifty male SD rats were randomly assigned to the sham group ( $n = 15$ ), the MI group ( $n = 20$ ), and the MI plus renal denervation group ( $n = 15$ ). MI was established through thoracotomic ligation of the anterior descending artery. Renal denervation was achieved by laparotomic stripping of the renal arterial adventitial sympathetic nerve, approximately 3 mm from the abdominal aorta. Left ventricular function and hemodynamics were measured several weeks following MI. The left ventricular systolic function of the MI group was significantly reduced and the systolic blood pressure (SBP) remarkably declined. In rats with MI treated with renal denervation, the left ventricular ejection fraction (EF), fractional shortening (FS) and SBP markedly improved compared with the MI group. However, heart rate and fibrosis decreased significantly. These findings suggest that renal denervation has therapeutic effects on post-MI cardiac dysfunction. These effects are associated with increased left ventricular ejection fraction (LVEF) and SBP, as well as reduced heart rate and fibrosis. This may represent a new approach to the treatment of post-MI remodeling and subsequent heart failure.

**Keywords:** renal denervation, myocardial infarction, heart failure

### Introduction

Acute myocardial infarction (MI) results in low blood pressure and loss of myocardial tissue. This leads to the activation of a series of neurohormonal factors, such as the sympathetic nervous system (SNS), the renin-angiotensin-aldosterone system (RAAS) and endothelin. These factors increase the heart rate, myocardial contractility and vasoconstriction to help maintain hemodynamic stability. Of these mechanisms, chronic activation of the SNS, which will lead to left ventricular remodeling and left ventricular dysfunctions, is the main mechanism of post-MI heart failure<sup>[1-2]</sup>.

In recent years, some clinical trials have demonstrated that renal denervation (RD) improved cardiac function in patients with resistant hypertension<sup>[3]</sup>. Furthermore, increasing data have revealed that blockade of the SNS and RAAS has therapeutic effects on heart failure<sup>[4-5]</sup>. This suggests that RD may have great potential in the treatment of heart failure and diseases related to chronic sympathetic activation.

To our knowledge, there are few animal studies which have confirmed the efficacy of RD intervention in MI-induced cardiac dysfunction or remodeling. Whether or not blunting the SNS in heart failure, which may be an initial adaptive response, will translate into a long-term

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Received 19 December 2014, Revised 5 February 2015, Accepted 5 May 2015, Epub 10 June 2015

CLC number: R54, Document code: A

The authors reported no conflict of interest.

benefit needs further research. Thus, we initiated this study to examine the effects of RD on post-MI cardiac systolic function in rats and the mechanism involved.

## Materials and methods

### Ethics statement

The experimental design and implementation were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (National Institutes of Health publication No. 85-23, revised 1985). The Animal Use and Management Ethics Committee of the First Affiliated Hospital of Nanjing Medical University approved the study protocol.

### Animals

Fifty male SD rats (SPF level, weighing 180–230 g) were obtained from the Experimental Animal Center of Jiangsu Province. These rats were housed with five rats per cage at a temperature of 22–25°C in a 50–70% humidity controlled room with a 12-hour light/dark cycle. A standard rat diet including tap water was given *ad libitum* throughout the experimental period.

### Groups

A complete randomization method was used and CHISS statistical software was used to generate random numbers. The rats were numbered in the ascending order and randomly assigned to three groups, the sham group ( $n = 15$ ), the MI group ( $n = 20$ ), and the MI+RD group ( $n = 15$ ). Fourteen rats did not survive surgery and each group ultimately contained 12 rats.

### Induction of myocardial infarction

Rats were endotracheally intubated and mechanically ventilated (Harvard Apparatus, USA) with supplemental oxygen under combined intraperitoneal anesthesia (ketamine, 50 mg/kg; diazepam, 5 mg/kg). The heart was exposed through a thoracotomy at the left fourth intercostal space, and the left anterior descending coronary artery (LAD) was ligated by a 7-0 silk suture together with a small amount of myocardial tissue approximately 1–2 mm from its origin. After ligation, lungs were fully inflated by positive end-expiratory pressure. The chest cavity, muscles, and skin were sutured in 3 layers. The surface of the heart turning white intraoperatively and ST segment elevation post operation signified a successful model. The rats were given pethidine (10 mg/day) and penicillin (80,000 U/day) intramuscularly for 3 days postoperatively. The sham group was subjected to an operation that used a similar procedure without coronary ligation. The perioperative mortality was approximately 40% in rats submitted to coronary artery ligation.

### Renal denervation

Two days after induction of myocardial infarction, bilateral RD was performed under intraperitoneal administration of a mixture of ketamine (50 mg/kg) and diazepam (5 mg/kg). Bilateral flank incisions were made, and RD was performed surgically by stripping the adventitia of the renal arteries at about 3 mm from the abdominal aorta for 1–2 cm and dissecting all visible renal nerve bundles, and coating the vessels with a solution of 10% phenol in ethanol. We evaluated the effects of RD by electrically stimulating (Grass S48 nerve stimulator, 15 V, 0.2 ms, 10 Hz) the renal sympathetic nerve at the proximal renal artery for 10–30 seconds before and after RD, respectively. In normal rats, electrical stimulation can cause the blood pressure to increase 5–10 mmHg, the heart rate (HR) to increase 8–15 bpm, and the kidney to become pale in color. When electrically stimulated after RD, sympathetic effects were absent with no apparent changes in blood pressure, HR heart rate, or kidney color.

### Echocardiography and hemodynamic assessment

Four weeks after MI induction, transthoracic echocardiography was performed under ether anesthesia using a cardiovascular ultrasound system (VisualSonics Vevo2100, Canada) with a 10-MHz linear-array transducer. The cardiac long-axis and short-axis views were obtained in the 2-dimensional mode, and M-mode tracings were recorded. End-diastolic and end-systolic LV internal dimensions and fractional shortening (FS) and ejection fraction were determined from at least three consecutive cardiac cycles. Following echocardiography, blood pressure and heart rate were recorded using a computerized, non-invasive tail-cuff system named Visitech BP-2000 Blood Pressure Analysis System™ (Visitech Systems, Apex, NC, USA). An observer who was blinded to the treatment performed all measurements.

### Tissue section preparation

After hemodynamic evaluation, the hearts were arrested in diastole by intravenous administration of 2 mol/L KCl, rapidly excised and washed in ice-cold saline. They were then dried with filter paper and weighed using an electronic scale to obtain the left ventricular mass (including the interventricular septum). The left ventricular mass index was calculated and the left ventricular infarct area ratio was determined. The myocardium was sliced into three blocks, each 4–5 mm thick and then fixed in 4% buffered neutral formalin at room temperature for 8–12 hours. The formalin-fixed tissue was embedded in paraffin wax and cut into 5- $\mu$ m sections before use. Three tissue blocks (base,

**Table 1** Baseline data of the study animals before myocardial infarction

	Sham	MI	MI+RD
Weight(g)	209.98 ± 10.72	199.63 ± 10.6	201.93 ± 16.11
SBP(mmHg)	132.94 ± 9.67	129.57 ± 6.1	127.76 ± 10.0
DBP(mmHg)	109.91 ± 6.32	105.65 ± 5.45	107.91 ± 10.5
HR(bpm)	379.19 ± 41.24	370.76 ± 37.54	375.57 ± 44.35
LVM(mg)	615.37 ± 33.84	575.12 ± 35.94	581.87 ± 35.99
LVEDV(μl)	324.54 ± 23.76	301.38 ± 21.45	317.03 ± 27.50
LVESV(μl)	103.07 ± 18.37	103.53 ± 13.59	102.13 ± 18.12
EF(%)	68.41 ± 3.62	65.74 ± 2.09	67.95 ± 2.8
FS(%)	39.67 ± 2.86	37.4 ± 1.55	39.24 ± 2.32

MI, myocardial infarction; RD, renal denervation; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVM, left ventricular mass; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; EF, ejection fraction; FS, fractional shortening; \* $P < 0.05$  vs. MI, # $P < 0.05$  vs. sham

mid-region, and apex) were used for histological examination and mean values were obtained for each heart.

### Histological analysis

Paraffin-embedded sections (5 slices/block) were stained with hematoxylin-eosin for morphologic examination or Masson's trichrome for assessment of interstitial fibrosis. To determine whether RD influences collagen fiber accumulation after infarction, we randomly selected 5 fields per slice and calculated the fibrosis area. The morphologic parameters and fibrosis area were measured using Image Pro Plus (Media Cybernetics), by a blinded observer.

### Statistical analysis

All values are expressed as mean ± SD. Analysis of all data was performed with the use of SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as  $P < 0.05$ . Group comparisons were made with analysis of variance (ANOVA), followed by the LSD test to identify differences among various group

## Results

### Effect of RD on cardiac systolic function, body weight and left ventricular mass index after MI

There was no significant difference in weight, blood pressure, heart rate and echocardiographic parameters for each group at baseline before MI (See **Table 1**).

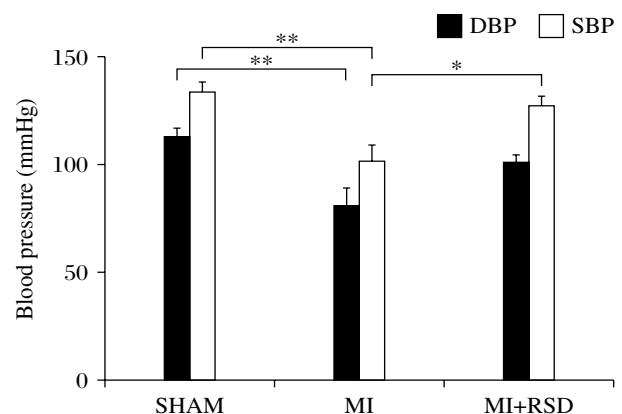
Four weeks post MI, EF and FS of the MI group were significantly reduced ( $49.40\% \pm 3.41\%$  and  $26.48\% \pm 2.11\%$  versus  $67.34\% \pm 8.10\%$  and  $39.49 \pm 6.79\%$ ,  $P < 0.01$ ). left ventricular end-systolic volume (LVESV) was significantly increased ( $297.98 \pm 64.24 \mu\text{L}$  versus  $154.36 \pm 73.82 \mu\text{L}$ ,  $P < 0.01$ ). EF and FS in the MI plus RD group, compared with the MI group,

were significantly increased, but lower than normal ( $P < 0.05$ ). LVESV in the MI plus RD group, compared with the MI group, was significantly decreased, but higher than normal ( $P < 0.05$ ). There was no significant difference in weight and left ventricular mass index for each group 4 weeks post MI (**Table 2**).

### Effect of RD on hemodynamics

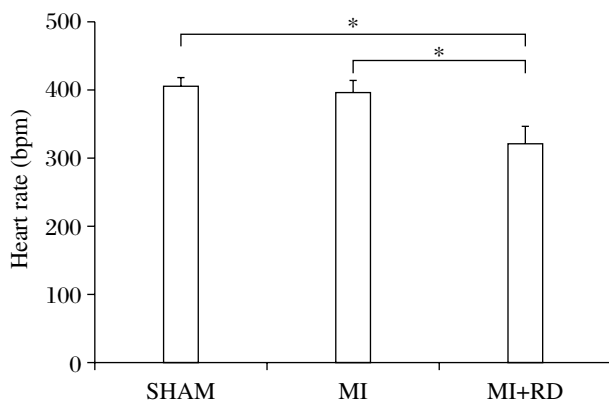
We observed that systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the MI group, compared with sham group, were significantly reduced ( $P < 0.01$ ). SBP in the MI plus RD group, compared with the MI group, was significantly increased ( $P < 0.05$ ). We also found that DBP rose, but did not show significant differences (**Fig. 1,2**).

As compared with the sham group and MI group, respectively, the HR in the MI plus RD group had a significant



**Fig. 1** Blood pressure four weeks after myocardial infarction.

The response of blood pressure to renal denervation four weeks after myocardial infarction among the three groups. SHAM ( $n = 12$ ), rats with renal innervation but without myocardial infarction; MI ( $n = 12$ ), rats with myocardial infarction but without renal denervation; MI+RD ( $n = 12$ ), rats with myocardial infarction and renal denervation. MI, myocardial infarction; RD, renal denervation; DBP, diastolic blood pressure; SBP, systolic blood pressure. \*\* $P < 0.01$  \* $P < 0.05$ .



**Fig. 2 Heart rate four weeks after myocardial infarction.** The response of heart rates to renal denervation four weeks after myocardial infarction among the three groups. SHAM ( $n = 12$ ), rats with renal innervation but without myocardial infarction; MI ( $n = 12$ ), rats with myocardial infarction but without renal denervation; MI+RD ( $n = 12$ ), rats with myocardial infarction and renal denervation. MI, myocardial infarction; RD, renal denervation. \* $P < 0.05$ .

decrease ( $P < 0.05$ ), but there was no significant difference between the MI group and the sham group (Fig. 2).

### Effect of RD on morphology and fibrosis

Masson's trichrome staining for interstitial fibrosis in the border zone is shown in Fig. 3A-3C. The border zone was confirmed by H&E staining on serial sections. Fibrosis area was reduced in the MI plus RD compared with the MI group (Fig. 3D).

### Discussion

MI expansion, left ventricular dilation and compensatory myocardial hypertrophy in noninfarcted regions are known to be involved in ventricular remodeling after coronary occlusion. In our study, although there were no significant differences within the three groups at baseline before MI (Table 1), the MI group underwent significant changes in cardiac function and hemodynamics compared with the sham group after MI. However, changes

in LVMI were not statistically significant (Table 2). Our findings were similar to those of Byung-Hee *et al.*<sup>[6]</sup> and Pfeffer *et al.*<sup>[7]</sup>. Although there was no significant difference in LVMI between the MI and sham groups, the mean value of MI was higher than that of the sham group four weeks post MI. In clinical practice, obvious post-MI compensatory myocardial hypertrophy is rare. Perhaps the infarct/LV ratio and/or the time course have impact on the pathophysiological process. As previously described, the hypertrophy of myocardial fibers in non-infarcted and border zones was found in rats after two hours of coronary occlusion<sup>[6]</sup>. In moderate and large infarcts, as inflammation and edema developed, LV weight increased in the early stage and then progressively decreased as a thin scar formed; the values then returned to normal as a result of compensatory hypertrophy of the residual myocardium<sup>[7]</sup>. In Hu *et al.*'s study, the findings were also similar; there was no significant change in LVMI one month post MI<sup>[8]</sup>.

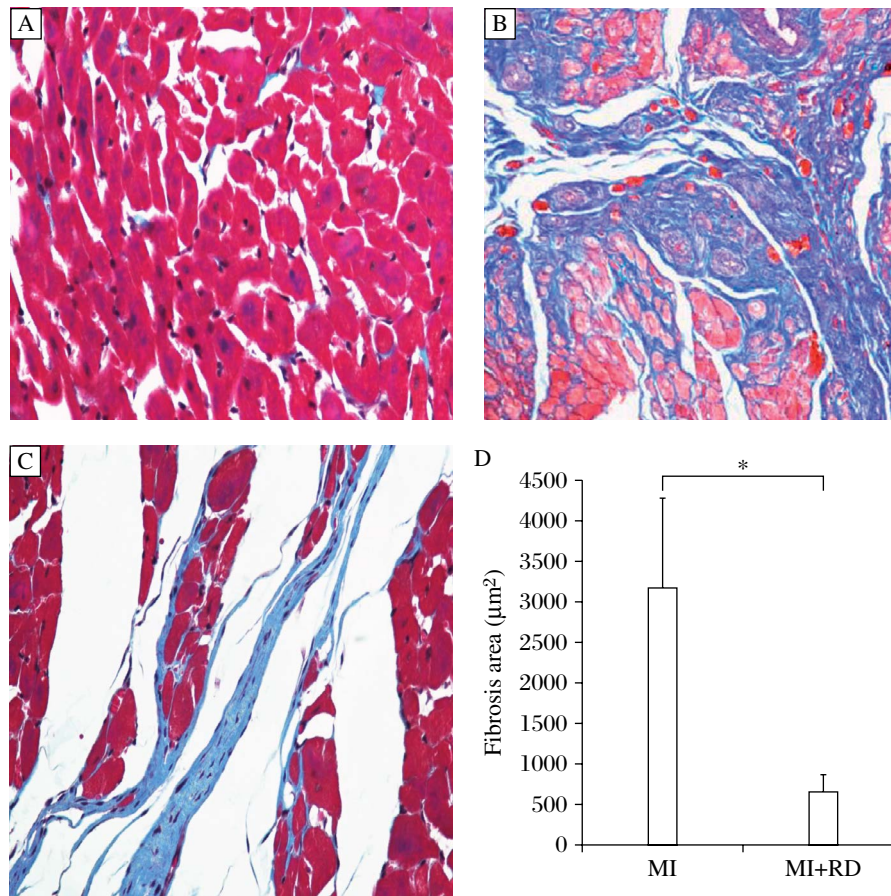
According to previous studies, an increase in sympathetic activity after MI might contribute to the progression of heart failure and, consequently, increased mortality<sup>[9]</sup>. Clinical studies have demonstrated that adrenergic receptor blockade is beneficial in heart failure due to decreased cardiac sympathetic stress that could reduce myocardial oxygen consumption, myocardial damage, and myocardial cell apoptosis<sup>[10-12]</sup>. In addition, angiotensin-convertase inhibition, which might reduce both volume and pressure overload, attenuates ventricular remodeling post MI<sup>[13]</sup>. The present study demonstrated that RD performed after myocardial infarction can lessen the deterioration of LV systolic function, including significantly increased EF and FS, while LVESV decreased significantly compared with the MI group (Table 2). Smaller LVESV in MI rats with RD due to the blocking of the sympathetic system and RAAS by RD may be an important component in the prevention of ventricular remodeling and improvement of LV systolic function post MI. Since reduced cardiac filling pressure without a concomitant reduction in

**Table 2 Body weight, left ventricular mass index and echocardiographic parameters 4 weeks after myocardial infarction**

	Sham	MI	MI+RD
Weight(g)	411.67 ± 8.33	395.14 ± 18.25	426.20 ± 44.51
LVMI(mg/g)	2.69 ± 0.24	2.91 ± 0.22	2.62 ± 0.36
LVM(mg)	1054.18 ± 284.24	1151.41 ± 126.12	1124.55 ± 242.74
LVEDV(μl)	461.04 ± 118.81	584.99 ± 95.42	472.61 ± 103.44
LVESV(μl)	154.36 ± 73.82	297.98 ± 64.24###	184.94 ± 73.58*
EF(%)	67.34 ± 8.10	49.40 ± 3.41##	61.82 ± 7.17*
FS(%)	39.49 ± 6.79	26.48 ± 2.11##	35.03 ± 4.96*

MI, myocardial infarction; RD, renal denervation; LVMI, left ventricular mass index; LVM, left ventricular mass; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; EF, ejection fraction; FS, fractional shortening; \* $P < 0.05$  vs MI, ## $P < 0.01$  vs sham





**Fig. 3** RD reduces interstitial fibrosis in the peri-infarct border zone 4 weeks after myocardial infarction. Masson's trichrome staining to detect interstitial fibrosis in the border zone of the sham group (A), MI group (B) and MI plus RD group (C). D, Semi-quantification of interstitial fibrosis expressed as fibrosis area in the border zone of the MI group and MI plus RD group. MI, myocardial infarction; RD, renal denervation.  $n = 3$  per group.  $*P < 0.05$  Bars = 20 µm.

arterial pressure lowered cardiac norepinephrine spill-over in heart failure<sup>[14]</sup>, cardiac adrenergic activity may be less in MI rats with RD than in rats with innervation because of lower LV end-diastolic pressure in RD rats in the present study; however, the degree of decrease failed to reach significant differences.

RD has been proven to be safe and effective in reducing SBP and DBP in patients with moderate to severe resistant hypertension<sup>[15-17]</sup>. However, data on the effect of RD on blood pressure in rats with post-MI heart failure are scarce. The present study has demonstrated that SBP and DBP in the MI rats significantly declined, compared with the sham group, at 4 weeks post MI. The results are consistent with previous animal studies and the established pathogenic mechanism of chronic heart failure<sup>[18-20]</sup>. Interestingly, contrary to the findings in humans, we found that RD could significantly increase SBP in rats with post-MI heart failure, but it had no significant impact on DBP. In regard to this difference, we speculated that the effect of RD on BP laid on the baseline values. In other words, rats with higher BP at baseline would experience a significant

decrease. Rats with lower BP at baseline tended to have an increase in BP after RDN. These differential effects may be attributable to the complex sympathetic-vagal interactions and/or the improvement of cardiac systolic function in rats with post-MI heart failure.

Elevated HR has been identified in previous studies as a predictor of poor outcome in patients with hypertension, coronary heart disease, myocardial infarction, and chronic heart failure (CHF)<sup>[21]</sup>. High HR contributes to the pathogenesis of vascular and myocardial diseases and is associated with a high prevalence of comorbidities<sup>[22-23]</sup>. Moreover, HR reduction has become a major therapeutic target in cardiovascular diseases. The Systolic Heart Failure Treatment with the I(f) Inhibitor Ivabradine Trial (SHIFT) has shown a reduction by 18% of cardiovascular death and hospitalization for heart failure in patients with systolic heart failure<sup>[24-25]</sup>. Our findings indicate that the reduction of sympathetic activity by RD leads to lower heart rates in rats with post-MI heart failure which may be beneficial, particularly in CHF, where heart rate and the autonomous dysfunction play a major pathophysiological role<sup>[25-26]</sup>.

Finally, when compared with the sham group, MI+RD group rats had slight increases in fibrosis, but significantly decreased fibrosis when compared with the MI group. The fibrosis area is also significantly smaller in MI+RD group than that in MI group (**Fig. 3**). Fibrosis is traditionally viewed as a phenomenon secondary to myocyte death and/or hypertrophy in response to injurious stimuli, and is a key contributor to the cardiac remodeling that occurs post-MI. Cardiac fibroblasts are the most prevalent cell type in the heart and play a key role in regulating normal myocardial function and in the adverse myocardial remodeling that occurs with hypertension, myocardial infarction and heart failure. As the main cellular effectors in matrix remodeling, and as important modulators of the inflammatory and reparative response, fibroblasts are promising therapeutic targets in cardiac remodeling post myocardial infarction. Considering our study, we propose the RD procedure would be a new therapy for anti-fibrosis after MI, and may improve cardiac function in acute MI.

The study might have some limitations. First, the size of the sample was small and the standard deviation too large. This might account for the lack of significant differences in LVMI between MI and MI+RD rats in the present study.

Second, due to lack of detection techniques and experimental scales, we did not measure indices such as blood or tissue norepinephrine or  $\beta$  receptors, which reflect the activity of the sympathetic nervous system and the reconstruction of the sympathetic nervous system. Blood content changes of RAAS components were not measured either. Besides BNP, we were also not able to measure indices such as endothelin. These indices are very important to RD research in post-MI heart failure.

Finally, after it was confirmed that RD improves systolic function and left ventricular remodeling in rats with post-MI heart failure, an in-depth study is warranted in further studies.

In conclusion, our study demonstrates that RD has a clear therapeutic effect on post-MI cardiac function through increasing cardiac systolic function and lessening left ventricular remodeling, with the effect lasting at least 4 weeks. However, there were no significant differences between the MI group and MI+RD group as LVMI and DBP were considered.

Based on previous studies, it was hypothesized that RD could prevent chronic renal afferent and efferent sympathetic activation in heart failure, thus blocking central sympathetic activation and reducing overall activity of the sympathetic nervous system. Inhibiting chronic activation of the sympathetic nervous system by blocking local sympathetic nerves such as renal sympathetic nerves leads to RD having the same effect

as sympathetic blockade medications on improving cardiac sympathetic system reconfiguration. However, unlike sympathetic blockade medications, RD could avoid a number of adverse reactions such as decreased cardiac contractility. Additionally, patients with severe renal insufficiency will have to avoid using the-blockers that have to be excreted by the kidneys. This highlights the importance of RD as a new non-drug treatment that blocks the sympathetic nervous system in heart failure.

However, previous basic and clinical studies indicate that surgical renal denervation causes great trauma, and that there are many complications or adverse effects related to the procedure itself or neurovascular damage, the effects of RD are complete and precise<sup>[27-28]</sup>. Many clinical studies have confirmed that RD via percutaneous radiofrequency catheter ablation is a minimally invasive procedure and an effective RD method with few complications or adverse effects after long-term observation<sup>[29-30]</sup>. The problem is that there is difficulty ensuring full effective ablation, and previous animal studies have shown neural regeneration<sup>[28]</sup>. If percutaneous catheters do not completely ablate the nerve, it could shorten the effective duration of RD. Further understanding of RD by radiofrequency ablation is needed.

### Acknowledgement

This study was supported by a project of Jiangsu Provincial Department of Health (H201302), which had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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