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Investigation of the mechanism underlying the antihypertensive effect of catheter-based radiofrequency renal sympathetic denervation in hypertensive dogs

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Abstract. The present study aimed to assess the antihypertensive efficacy and safety of catheter-based radiofrequency renal sympathetic denervation (RSD) in hypertensive dogs. Furthermore, the study investigated the possible antihypertensive mechanism of radiofrequency RSD through measuring the postoperative serum concentrations of angiotensin II (AngII), nicotinamide adenine dinucleotide phosphate oxidase (NADPH-ox), malondialdehyde (MDA), nitric oxide (NO) and endothelial NO synthase (eNOS). A total of 12 beagles were randomly divided into the surgery (n=6) and the sham-surgery groups (n=6). The hypertension model was established using a high-fat diet. The surgery group received catheter-based radiofrequency RSD, while the sham-surgery group only received renal arteriography. Blood pressure was measured prior to the surgery and 3 days, 1 and 2 weeks, and 1, 2 and 3 months after the surgery. The serum concentrations of AngII, NADPH-ox, MDA, NO and eNOS were measured prior to the surgery and 1 week, and 1 and 3 months after the surgery. Following the establishment of the model, the systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP) of the surgery and the sham-surgery groups were all significantly increased above the baseline ($P<0.05$), but there was no significant difference between the two groups. SAP, DAP and MAP in the surgery group at 1 and 3 months after the surgery were significantly decreased compared to the levels measured prior to the surgery and those in the sham-surgery group ($P<0.05$). Three months after the surgery, the serum creatinine level was normal and renal arteriography did not show renal artery stenosis. Compared to those measured prior to the surgery, the concentrations of serum AngII, NADPH-ox and MDA in the surgery group at 1 week, and 1 and 3 months after the surgery were decreased, while the concentrations of serum NO and eNOS were increased ($P<0.05$). The above indicators measured at the same time points demonstrated statistically significant differences between the surgery and the sham-surgery groups ($P<0.05$). In conclusion, catheter-based radiofrequency RSD may inhibit the renin-angiotensin system and the oxidative stress response, as well as improve vascular endothelial function, thus significantly reducing blood pressure through the reduction of sympathetic activity in hypertensive dogs.

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Key words: hypertension, catheter-based ablation, renal sympathetic denervation, renin-angiotensin system, oxidative stress, endothelial function

Introduction

With the rapid development of the catheter technique and taking into account the pivotal function of renal sympathetic nerves in hypertension, the study by Krum *et al* (1,2) first applied catheter-based radiofrequency renal sympathetic denervation (RSD) for the treatment of patients with refractory hypertension and obtained a positive antihypertensive efficacy. As a new antihypertensive technique, radiofrequency RSD became a new treatment of hypertension; however, its specific antihypertensive effect and the underlying mechanism require further study (2,3). In addition, other than the significant inhibition of renal sympathetic activity, the effects of radiofrequency RSD on the renin-angiotensin system (RAS), oxidative stress and vascular endothelial function remain unclear. In the present study, a hypertensive dog model was established by administering a high-fat diet and the antihypertensive effect and safety of radiofrequency RSD were validated using these hypertensive dogs. The changes in serum angiotensin II (AngII), nicotinamide adenine dinucleotide phosphate oxidase (NADPH-ox), serum malondialdehyde (MDA), nitric oxide (NO) and endothelial NO synthase (eNOS) were detected to investigate the possible antihypertensive mechanisms of radiofrequency RSD.

Materials and methods

Animals. A total of 12 same-strain qualified beagles, aged 10-12 months and including 6 for each gender, were purchased from the Shanghai Experimental Animal Center (Shanghai, China). Animals were purchased 2 weeks prior to the experiment and were housed in a single cage in the Xiangya Animal Center of Central South University (Changsa, China). After 1 week of habituation, the 12 beagles were randomly divided into the surgery (n=6) and sham-surgery groups (n=6). Following the establishment of the hypertension model, radiofrequency RSD was performed in the surgery group; the sham-surgery group only received renal arteriography. The study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University.

Establishment of the hypertension model. Animals were provided a high-fat diet for 3 months, which consisted of 150 g basic feed/animal with the addition of edible lard at 0.3-0.4 kg/animal. The basic feed was common dog food (Xingtai Paide Pets Food Co., Ltd., Nanhe, Hebei, China) which contained 23% crude protein, 4.9% crude fiber, 10% water, 9.2% ash, 1-3% calcium, 0.8% total phosphorus, 0.29% methionine, 11,000 IU/kg vitamin A, 1,000 IU/kg vitamin D and 500 IU/kg vitamin E. Edible lard (Sichuan Green Island Food Co., Ltd., Chengdu, Sichuan, China) was purchased from the grain and oil market. Following successful establishment of the model and the application of radiofrequency RSD, animals in the surgery and sham-surgery groups were fed a diet containing 0.1 kg/day fat to maintain their body weight (4).

Monitoring of pressure. The tail arterial pressure of the dogs in the awake condition was measured using a BP-10E intelligent non-invasive animal blood pressuremeter (Sofron Beijing Inc., China). The dogs were placed on a fixator and when they were completely calm, sleeves were placed on the root of their tails. Following appropriate setup of the parameters associated with the blood pressure meter, the instrument automatically read the systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and heart rate (HR). Prior to the establishment of the model, blood pressure levels were measured twice per week. After the measured blood pressure and HR values were stable, which were used as the baseline values, the establishment of the model began. The measurement time points included before model establishment and 2, 4, 6 and 12 weeks after model establishment. The blood pressure readings gradually stabilized after 12 weeks and radiofrequency RSD was performed. Blood pressure was measured before the surgery and 3 days, 1 and 2 weeks, and 1, 2 and 3 months after the surgery.

Radiofrequency RSD intervention. The dogs were fasted prior to the surgery. The skin areas at the back and bilateral femoral artery area of the dogs were prepared accordingly, followed by an intramuscular injection of Zoletil® (7 mg/kg; Virbac, Carros, France) and Sumianxin (0.1 mg/kg; Jilin Shengda Animal Drug Co., Ltd., Yanbian, Jilin, China) for anesthetization. Following successful anesthesia, the dogs were immobilized on the surgery table in the supine position. An ablation electrode was placed on the back and connected to a radiofrequency ablation device (IBI, St. Jude Medical, Inc., St. Paul, MN, USA). The temperature was controlled at ~55˚C and the energy was set at 80 W. The right femoral artery area was conventionally disinfected and a guiding catheter was inserted through the femoral artery for renal arteriography and renal artery positioning as the blood pressure was monitored. Three to four ablation sites were selected from each site and a spiral shape local ablation was performed (5F IBI radiofrequency ablation catheter; St. Jude Medical). Each spot was ablated for 120 sec. Blood pressure was monitored following completion of the surgery. Sterile gauze was pressed on the puncture site to stop the bleeding and penicillin was administered following the surgery to prevent infection. Blood pressure and HR were measured in the morning during the awake and non-feeding conditions at 3 days, 1 and 2 weeks, and 1 and 3 months after the surgery. Renal arteriography was performed again at 3 months after the surgery. The sham-surgery group only received renal arteriography (1,4).

Measurement of neuroendocrine factors. A total of 3 ml of blood was collected from the great saphenous vein of all the dogs at baseline (before model establishment), before the surgery (after model establishment) and at 1 week, and 1 and 3 months after radiofrequency RSD. Blood samples were stored in anticoagulant tubes at 4˚C. Subsequently, the samples were centrifuged in a refrigerated centrifuge at 2,780 x g for 15 min to separate the serum (Xiangyi Centrifuge Instrument Co., Ltd., Changsha, Hunan, China) and the obtained serum samples were stored in a -80˚C freezer (Meiling Freezer Technology Co., Ltd., Hefei, Anhui, China) for subsequent tests. The concentration of AngII was measured using a radioimmunoassay (the reagent kit was provided by Beijing Chemclin Biotech Co., Ltd., Beijing, China). The concentration of MDA was measured using the thiobarbituric acid method (the reagent kit was provided by the Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). The serum NO concentration was determined using the nitrate reductase method (the reagent kit was provided by the Nanjing Jiancheng Bioengineering Institute). The serum eNOS concentration was determined using the chemical method (the reagent kit was provided by the Nanjing Jiancheng Bioengineering Institute). The concentration of serum NADPH-ox was measured using ELISA (the reagent kit was provided by the Shanghai BlueGene Biotech Co., Ltd., Shanghai, China).

Statistical analysis. SPSS version 17.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses. Measurement data are reported as the mean ± standard deviation. The major data were subjected to the normality test and the test of homogeneity of variance. The comparison of means between two groups was performed using the t-test and the comparison among multiple groups was performed using one-way analysis of variance. $P<0.05$ was considered to indicate a statistically significant difference and $P<0.01$ indicated a strongly significant difference.

Results

Baseline condition of animals in the two groups. The comparison of body weight, HR, blood pressure and creatinine level between animals in the two groups did not reveal any significant differences ($P>0.05$) (Table I).

Table I. Comparison of the baseline condition between the surgery and sham-surgery groups prior to establishment of the model.

Group	Weight, kg	HR, time/min	SAP, mmHg	DAP, mmHg	MAP, mmHg	Creatinine, μmol/l
Sham-surgery	13.12±1.41	129±10	129.22±6.95	76.44±5.09	94.00±4.91	81.9±10.4
Surgery	13.08±2.43	127±13	122.89±4.84	75.64±6.09	93.39±4.66	82.4 ±12.1

Data are presented as mean ± standard deviation, n=6. HR, heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure.

Table II. Comparison of SAP, DAP and MAP levels prior to the surgery between the surgery and the sham-surgery groups.

	SAP, mmHg		DAP, mmHg		MAP, mmHg	
Group	Baseline	Post-model establishment	Baseline	Post-model establishment	Baseline	Post-model establishment
Sham-surgery	129.22±6.95	149.33±9.16[a]	76.44±5.09	83.56±2.52[a]	94.00±4.91	109.44±2.14[a]
Surgery	122.89±4.84	152.28±8.60[a]	75.64±6.09	82.56±3.58[a]	93.39±4.66	114.22±4.25[a]

[a]Compared to the baseline level in the same group, P<0.05. Data are presented as mean ± standard deviation, n=6. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure.

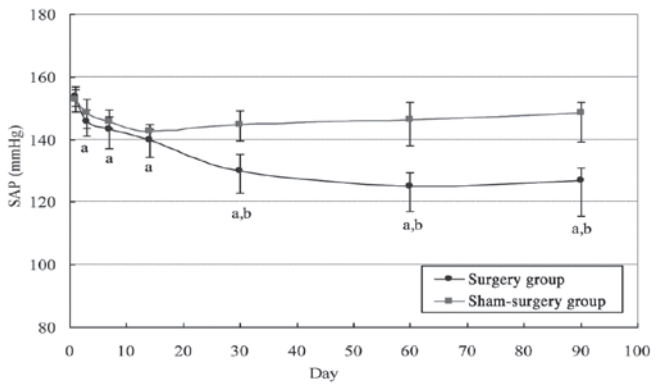

Figure 1. Changes in systolic arterial pressure (SAP) prior and subsequent to the surgery in the surgery and sham-surgery groups. [a]Compared to the baseline level in the same group, P<0.05; [b]compared to the sham-surgery group, P<0.05.

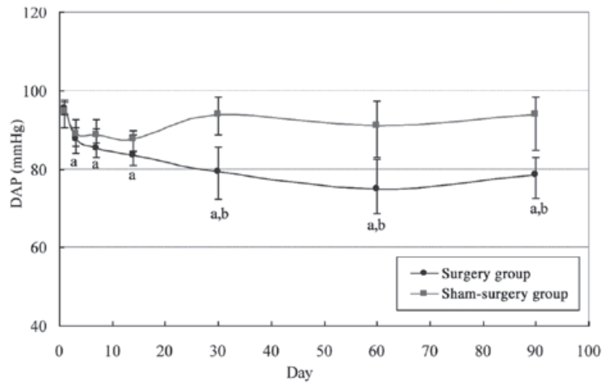

Figure 2. Changes in diastolic arterial pressure (DAP) prior and subsequent to the surgery in the surgery and sham-surgery groups. [a]Compared to the baseline level in the same group, P<0.05; [b]compared to the sham-surgery group, P<0.05.

Comparison of SAP, DAP and mean arterial pressure (MAP) between animals in the two groups prior to establishing the model. Compared to those measured prior to model establishment, the levels of SAP, DAP and MAP of animals in the two groups increased significantly following model establishment (P>0.05), indicating that the model was successfully established and there was no difference between the two groups (Table II).

Changes in blood pressure following radiofrequency RSD. The levels of SAP, DAP and MAP in the surgery group at each time point following the surgery were significantly decreased and showed significant differences from those obtained prior to the surgery (P<0.05) (Figs. 1-3). In addition, the levels of SAP, DAP and MAP in the sham-surgery group within 2 weeks after the surgery also slightly decreased but did not

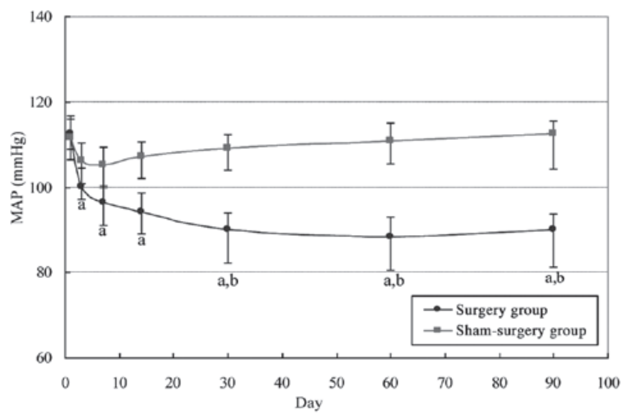

Figure 3. Changes in mean arterial pressure (MAP) prior and subsequent to the surgery in the surgery and sham-surgery groups. [a]Compared to the baseline level in the same group, P<0.05; [b]compared to the sham-surgery group, P<0.05.

Table III. Comparison of the serum concentrations of angiotensin II (AngII) at each time point between the surgery and sham-surgery groups.

	AngII concentration, ng/ml				
Group	Baseline	Post-model establishment (prior to surgery)	1 week post-surgery	1 month post-surgery	3 months post-surgery
Sham-surgery	51.76±10.77	120.77±17.30[a]	123.61±11.78[a]	124.60±13.20[a]	126.30±12.92[a]
Surgery	56.69±7.17	121.54±18.09[a]	95.18±7.73[a-c]	89.08±8.43[a-c]	90.11±11.10[a-c]

[a]Compared to the baseline level, P<0.05; [b]compared to the concentration prior to the surgery, P<0.05; [c]compared to the sham-surgery group at the same period, P<0.05. Data are presented as mean ± standard deviation, n=6.

Table IV. Comparison of the serum concentrations of nicotinamide adenine dinucleotide phosphate oxidase (NADPH-ox) at each time point between the surgery and sham-surgery groups.

	NADPH-ox concentration, ng/ml				
Group	Baseline	Post-model establishment	1 week post-surgery	1 month post-surgery	3 months post-surgery
Surgery	1.25±0.13	1.67±0.16[a]	1.37±0.19[a-c]	1.35±0.19[a-c]	1.35±0.18[a-c]
Sham-surgery	1.30±0.11	1.63±0.14[a]	1.60±0.28[a]	1.63±0.23[a]	1.63±0.22[a]

[a]Compared to the baseline level, P<0.05; [b]compared to the concentration prior to the surgery, P<0.05; [c]compared to the sham-surgery group at the same period, P<0.05. Data are presented as mean ± standard deviation, n=6.

Table V. Comparison of the serum malondialdehyde (MDA) concentrations at each time point between the surgery and the sham-surgery groups.

	MDA concentration, nmol/ml				
Group	Baseline	Post-model establishment	1 week post-surgery	1 month post-surgery	3 months post-surgery
Surgery	5.29±1.33	14.35±1.27[a]	11.55±4.10[a-c]	11.24±2.98[a-c]	11.20±3.16[a-c]
Sham-surgery	5.11±1.50	15.00±1.88[a]	15.05±1.96[a]	15.26±2.79[a]	15.22±1.81[a]

[a]Compared to the baseline level, P<0.05; [b]compared to the concentration prior to the surgery, P<0.05; [c]compared to the sham-surgery group at the same period, P<0.05. Data are presented as mean ± standard deviation, n=6.

show significant differences from those in the surgery group (P>0.05). However, at 1, 2 and 3 months after surgery, the comparison of SAP, DAP and MAP between the surgery and the sham-surgery groups demonstrated statistically significant differences (P<0.05).

Changes in the concentration of AngII following radiofrequency RSD. The comparison of the baseline serum concentrations of AngII between the sham-surgery and the surgery groups did not reveal significant differences (P>0.05) (Table III). Following model establishment, the serum AngII concentrations in the two groups were significantly increased compared to the baseline levels (P<0.05), with no difference between the two groups (P>0.05). The serum concentrations of AngII at 1 week, and 1 and 3 months after radiofrequency RSD in the sham-surgery group did not change, whereas these levels were significantly decreased in the surgery group, with a significant difference from the baseline level and those observed prior to the surgery and in the sham-surgery group (P<0.05).

Changes in the serum concentrations of NADPH-ox and MDA following radiofrequency RSD. Comparison of the baseline serum concentrations of NADPH-ox and MDA between the surgery and sham-surgery groups did not reveal significant differences (P>0.05) (Tables IV and V). Following model establishment, the serum concentrations of NADPH-ox and MDA in these two groups significantly increased from the baseline level (P<0.05); however, there was no difference between these two groups (P>0.05). In the sham-surgery

Table VI. Comparison of the serum endothelial nitric-oxide synthase (eNOS) concentrations at each time point between the surgery and the sham-surgery groups.

	eNOS concentration, U/ml				
Group	Baseline	Post-model establishment	1 week post-surgery	1 month post-surgery	3 months post-surgery
Surgery	11.26±1.49	5.85±1.49[a]	8.86±2.33[a-c]	9.00±2.33[a-c]	9.00±2.04[a-c]
Sham-surgery	11.38±1.78	6.16±1.60[a]	6.19±2.00[a]	6.07±1.53[a]	6.13±1.59[a]

[a]Compared to the baseline level, P<0.05; [b]compared to the concentration prior to the surgery, P<0.05; [c]compared to the sham-surgery group at the same period, P<0.05. Data are presented as mean ± standard deviation, n=6.

Table VII. Comparison of the serum concentrations of nitric oxide (NO) at each time point between the surgery and the sham-surgery groups.

	NO concentration, μmol/l				
Group	Baseline	Post-model establishment	1 week post-surgery	1 month post-surgery	3 months post-surgery
Surgery	95.37±6.69	52.45±8.65[a]	68.53±9.65[a-c]	69.13±7.83[a-c]	69.42±9.08[a-c]
Sham-surgery	95.36±8.08	55.30±8.56[a]	57.31±9.36[a]	52.68±5.51[a]	52.66±11.78[a]

[a]Compared to the baseline level, P<0.05; [b]compared to the concentration prior to the surgery, P<0.05; [c]compared to the sham-surgery group at the same period, P<0.05. Data are presented as mean ± standard deviation, n=6.

group, the serum concentrations of NADPH-ox and MDA at 1 week, and 1 and 3 months after the surgery did not change. By contrast, these concentrations significantly decreased in the surgery group, showing statistically significant differences in comparison to the baseline levels and those observed prior to the surgery and in the sham-surgery group (P<0.05).

Changes in serum eNOS and NO concentrations following radiofrequency RSD. Comparison of the baseline serum concentrations of eNOS and NO in the surgery and the sham-surgery groups did not demonstrate statistically significant differences (P>0.05) (Tables VI and VII). Following model establishment, the serum concentrations of eNOS and NO in these two groups were significantly increased compared to the baseline level (P<0.05); however, there was no difference between these two groups (P>0.05). The serum concentrations of eNOS and NO in the sham-surgery group at 1 week, and 1 and 3 months after surgery did not change. By contrast, the concentrations in the surgery group significantly decreased and showed significant differences in comparison to the baseline levels and those observed prior to the surgery and in the sham-surgery group (P<0.05).

Safety of radiofrequency RSD. There were no deaths in the surgery or sham-surgery groups. Three dogs developed hematomas at the puncture sites, although following pressure depressing and bandaging, there were no sequelae. During ablation, two dogs experienced renal artery spasms and their symptoms were relieved 10 min after the injection of nitroglycerin. Radiography was performed immediately following the radiofrequency RSD surgery and there was no evidence of renal artery dissection. The serum creatinine level was examined 3 days, 1 and 2 weeks, and 1 and 3 months after the surgery and the results were all normal. Additionally, renal arteriography was performed again at 3 months after the surgery and no stenosis was observed.

Discussion

Hypertension is a common cardiovascular disease and the leading risk factor for cardiovascular mortalities in China. This disease not only severely endangers human health but also creates a tremendous burden to the community. To thoroughly investigate the pathogenesis and treatment methods for hypertension, a number of hypertension experimental animal models have been established. Lohmeier *et al* (4) provided dogs a high-fat diet for 4 weeks, which led to increases in body weight that were 1.5 times greater than that of the control group and an increased MAP of 17±3 mmHg, indicating successful establishment of a hypertension model. This model is not only stable and has a high success rate but also possesses characteristics similar to human hypertension. In the present study, beagle dogs were selected as study subjects. Following feeding with a high-fat diet for 12 weeks, the blood pressure levels in the two groups of animals significantly increased (P<0.05), indicating that the hypertension model was established successfully. The increase in sympathetic nerve activity is the most important factor contributing to the elevation of blood pressure. The sympathetic nervous system can activate the RAS and AngII can activate the presynaptic angiotensin II type 1 receptor to promote the release of catecholamines, which can in turn indirectly activate sympathetic nerve activity to cause increased blood pressure and form a vicious cycle. As one of the most important active biological molecule in RAS, AngII plays significant roles in vasoconstriction and water-sodium retention and its level can partially reflect sympathetic nerve activity. The present study showed that the serum AngII levels in the two groups of dogs following successful model establishment significantly increased from the baseline level (P<0.05), although the serum AngII level in the sham-surgery group always maintained a higher level. These results suggested that activation of the RAS occurred in the high-fat-diet-induced hypertension model and that the increase in blood pressure in this dog model was significantly associated with activation of the RAS. The results of the study also showed that serum NADPH-ox and MDA levels increased, whereas eNOS and NO production significantly decreased in this dog model. The increase in NADPH-ox and MDA levels may reflect the activation of oxidative stress and the decrease in eNOS and NO typically reflects damage associated with vascular endothelial function. Therefore, these results indicate that oxidative stress and endothelial dysfunction may also play important roles in the development of hypertension in this dog model.

Blood pressure was measured at each time point following radiofrequency RSD and the results showed that within 2 weeks of the intervention the blood pressure of the surgery and sham-surgery groups decreased. The decrease in blood pressure in the surgery group was more evident, which may be associated with the influence of surgical strike and anesthetic agents. The blood pressure of the sham-surgery group gradually recovered to the preoperative level, whereas that in the surgery group gradually decreased, indicating that radiofrequency RSD had an antihypertensive effect that emerged as soon as 3 days after the surgery. The antihypertensive effect gradually increased with time and reached a relatively stable level 1 month after the surgery and this antihypertensive effect was maintained at 2 and 3 months after the surgery. In addition, the experimental data showed that the changing trends of SAP, DAP and MAP in the surgery group following the surgery were consistent with each other, indicating that radiofrequency RSD reduced SAP, DAP and MAP, consistent with previous international studies (5,6). However, the long-term antihypertensive effect of this procedure requires further observation. Radiofrequency RSD selectively transects renal sympathetic nerves to inhibit sympathetic nerve activity, thus affecting the release of catecholamines. In the study by Krum *et al* (1), the release of norepinephrine decreased by an average of 47% following radiofrequency RSD. Allen (7) also confirmed that renal denervation reduced renal sympathetic nerve activity, enhanced the functions of urinary sodium excretion and diuresis and decreased renin release. In addition, studies have shown that the levels of catecholamine, renin and AngII in the circulation can partially reflect sympathetic nerve activities. The present study showed that the serum AngII levels at 1 week, and 1 and 3 months after radiofrequency RSD were significantly decreased compared to those observed prior to the surgery (P<0.05); additionally, these levels paralleled the decrease in blood pressure, whereas these levels in the sham-surgery group continuously increased. These results confirmed that the decrease in blood pressure observed in hypertensive dogs was associated with RAS inhibition caused by the reduction of sympathetic activity.

NADPH-ox is an important oxidase in the body that consists of numerous subunits, including p22phox, gp91phox, p47phox and Rac2 (8). NADPH-ox is the main source of oxidative stress in the vascular system. A number of studies have shown that primary hypertension patients and spontaneously hypertensive rats have higher levels of NADPH-ox and produced a greater amount of reactive oxygen species (ROS) compared to the control group. As the detection of ROS is difficult, MDA was detected, which is more representative of the level of oxidative stress in the body (9). Oxidative stress and the sympathetic nervous system are closely associated. An increase in the ROS level can activate the RAS and sympathetic nerve system (10) and sympathetic nervous excitement can also increase the activity of NADPH-ox, possibly through influencing α1-adrenergic-receptor-mediated activation of the phospholipase C and protein kinase C pathways (11). If oxidative stress can be effectively inhibited during the pathogenesis of hypertension, hypertension may be effectively prevented and treated. Rafiq *et al* (12) found that radiofrequency RSD in rats with aortic regurgitation led to a reduction in renal norepinephrine, angiotensinogen and AngII, as well as an improvement in oxidative stress. The present study showed that in the high-fat diet-induced hypertension model, the AngII level decreased following radiofrequency RSD. In addition, the serum NADPH-ox and MDA levels were significantly decreased at each time point following the surgery compared to those measured prior to the surgery (P<0.05). Compared to the levels in the sham-surgery group, the levels of these factors at 1 and 3 months after the surgery were significantly decreased (P<0.05), indicating that blockade of the renal sympathetic nerves inhibited oxidative stress.

Vascular endothelial cells secrete various vasoactive substances, including NO, which is the most important vasodilator in the body. Studies have shown that damage to endothelial function is associated with a decrease in endothelium-dependent NO activity and thus decreased NO release can be used as a marker of endothelial injury. As a necessary enzyme for NO synthesis, eNOS shares certain trends with NO. For example, hemodynamic pressure changes caused by hypertension can reduce NOS activity and damage NO synthesis, thus resulting in endothelial dysfunction (13). Hypertension and eNOS are also closely associated. Kayhan *et al* (14) found that the E298D polymorphism of eNOS can increase the risk of hypertension and eNOS-knockout mice were also shown to develop hypertension (15). The present study showed that the serum levels of eNOS and NO in hypertensive dogs were significantly lower than those in the control group, suggesting that hypertensive dogs had damaged vascular endothelial function. One possible reason for this result was that the increased synthesis and secretion of AngII may have increased the NADH/NADPH-ox activity in the endothelium and vascular smooth muscle membrane, thus increasing vascular hydroxyl radicals to damage endothelium-dependent vasorelaxation. Vascular endothelial dysfunction is an early pathological change in numerous cardiovascular diseases. If the damaged vascular endothelial function can be improved along with the antihypertensive effect, the occurrence of complications of hypertension can be effectively postponed or prevented. Currently, it is generally believed that the mechanism underlying endothelial cell damage induced by hypertension is mediated by AngII. For example, AngII can cause endothelial dysfunction through the activation of oxidative stress and oxidative stress-dependent signaling pathways. The present study showed that the serum levels of eNOS and NO in the surgery group were significantly higher than those in the sham-surgery group at each time point following radiofrequency RSD, suggesting that damaged vascular endothelial function was improved by radiofrequency RSD. Additionally, the steadily decreased level of AngII may not only improve endothelial function through the reduction of blood pressure, but also through the relief of oxidative stress and vasoconstriction.

In conclusion, in the high-fat-diet-induced hypertension dog model, activation of the RAS, enhancement of oxidative stress and endothelial dysfunction may serve as mechanisms underlying the elevation of blood pressure. Radiofrequency RSD not only persistently reduced the levels of SAP, DAP and MAP in hypertensive dogs, but also reduced the serum concentrations of AngII, NADPH-ox and MDA and increased the levels of eNOS and NO. These results indicate that radiofrequency RSD may significantly reduce blood pressure in hypertensive dogs through the inhibition of the RAS and oxidative stress response and the improvement of vascular endothelial function by suppressing sympathetic activity. These results may provide an experimental and theoretical basis for the antihypertensive mechanism and target organ protection provided by catheter-based radiofrequency RSD.

The limitations of the study include the small samples size and insufficient postoperative observation time due to difficulties in the manipulation in large animals and the long-term antihypertensive effect of radiofrequency RSD. In addition, the mechanism underlying the antihypertensive effect of catheter-based radiofrequency RSD is complex and multifactorial; therefore, this topic requires further in-depth and long-term studies.

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References

1. Krum H, Schlaich M, Whitbourn R, *et al*: Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. Lancet 373: 1275-1281, 2009.
2. Krum H, Schlaich MP, Sobotka PA, *et al*: Percutaneous renal denervation in patients with treatment-resistant hypertension: final 3-year report of the Symplicity HTN-1 study. Lancet 383: 622-629, 2014.
3. Papademetriou V, Doumas M and Tsioufis K: Renal sympathetic denervation for the treatment of difficult-to-control or resistant hypertension. Int J Hypertens 2011: 196518, 2011.
4. Lohmeier TE, Iliescu R, Liu B, *et al*: Systemic and renal-specific sympathoinhibition in obesity hypertension. Hypertension 59: 331-338, 2012.
5. Symplicity HTN-1 Investigators: Catheter-based renal sympathetic denervation for resistant hypertension: durability of blood pressure reduction out to 24 months. Hypertension 57: 911-917, 2011.
6. Symplicity HTN-2 Investigators, Esler MD, Krum H, *et al*: Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. Lancet 376: 1903-1909, 2010.
7. Allen TR: Current status of lumbar sympathectomy. Am Surg 42: 89-91, 1976.
8. Babior BM: NADPH oxidase: an update. Blood 93: 1464-1476, 1999.
9. Maboudou P, Mathieu D, Bachelet H, *et al*: Detection of oxidative stress. Interest of GC-MS for malondialdehyde and formaldehyde monitoring. Biomed Chromatogr 16: 199-202, 2002.
10. Carlson SH and Wyss JM: Neurohormonal regulation of the sympathetic nervous system: new insights into central mechanisms of action. Curr Hypertens Rep 10: 233-240, 2008.
11. DiBona GF and Kopp UC: Neural control of renal function. Physiol Rev 77: 75-197, 1997.
12. Rafiq K, Noma T, Fujisawa Y, *et al*: Renal sympathetic denervation suppresses de novo podocyte injury and albuminuria in rats with aortic regurgitation. Circulation 125: 1402-1413, 2012.
13. Beevers G, Lip GY and O'Brien E: ABC of hypertension: the pathophysiology of hypertension. BMJ 322: 912-916, 2001.
14. Kayhan FE, Koldemir M, Cagatay P, *et al*: Prevalence of endothelial nitric oxide synthase E298D polymorphism in Turkish patients with essential hypertension. Diabetes Metab Syndr 7: 12-16, 2013.
15. Rees DD, Palmer RM and Moncada S: Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc Natl Acad Sci USA 86: 3375-3378, 1989.