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Original Article

Guizhi Decoction (桂枝汤) Inhibits Cholinergic Transdifferentiation by Regulating Imbalance of NGF and LIF in Salt-Sensitive Hypertensive Heart Failure Rats*

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ABSTRACT **Objective:** To observe the imbalance of anatomical and functional innervation factors of sympathetic nerves, nerve growth factor (NGF) and leukemia inhibitory factor (LIF), in salt-sensitive hypertensive heart failure rats and to explore the effects of treatment with Guizhi Decoction (桂枝汤) on sympathetic remodeling by inhibiting cholinergic transdifferentiation. **Methods:** SS-13^{BN} and Dahl salt-sensitive (DS) rats were divided into 3 groups: SS-13^{BN} group (control group, *n*=9), DS group (model group, *n*=9) and GS group (Guizhi Decoction, *n*=9). After 10 weeks of a high-salt diet, the GS group rats were given Guizhi Decoction and other two groups were given saline at an equal volume as a vehicle. After 4 weeks' intragastric administration, rats were executed to detect the relevant indicators. Echocardiography and plasma n-terminal pro-B type natriuretic peptide (NT-proBNP) levels were used to assess cardiac function. Noradrenaline (NA) levels in the plasma and myocardium were detected to evaluate the sympathetic function. NGF and LIF expression were detected in the myocardium by Western blot or quantitative real-time PCR. Double immunofluorescence or Western blot was used to detect tyrosine hydroxylase (TH), choline acetyltransferase (CHAT) and growth associated protein 43 (GAP43) in order to reflect anatomical and functional changes of sympathetic nerves. **Results:** DS group had anatomical and functional deterioration of sympathetic nerves in the decompensation period of heart failure compared with SS-13^{BN} group. Compared with the DS group, Guizhi Decoction significantly decreased the expression of LIF mRNA/protein (P<0.01), increased the expression of NGF (P<0.05 or P<0.01), enhanced the levels of TH$^+$/GAP43$^+$ and TH$^+$/CHAT$^+$ positive nerve fibers (P<0.01), and improved the protein expression of TH and GAP43 in left ventricle, but had no effect on CHAT (P>0.05). Guizhi Decoction inhibited inflammatory infiltration and collagen deposition of myocardial injury, increased the content of myocardial NA (P<0.05), reduced the plasma NA level (P<0.01), improved cardiac function (P<0.01), and improved weight and blood pressure to some extent (P<0.05), compared with DS group. **Conclusions:** Guizhi Decoction could inhibit cholinergic transdifferentiation of sympathetic nerves, improve the anatomical and functional denervation of sympathetic nerves, and delay the progression of decompensated heart failure. The mechanism may be associated with the correction of the imbalance of NGF and LIF.

KEYWORDS sympathetic, cholinergic transdifferentiation, nerve growth factor, leukemia inhibitory factor, Guizhi Decoction

Cardiac sympathetic system hyperactivity is a specific hallmark of neurohormonal imbalance in heart failure (HF).[1] Currently, it is widely known that inhibition of the sympathetic system can be an effective treatment for HF.[2-4] However, relevant reports indicated that anatomical and functional denervation of cardiac sympathetic nerves appeared during the process of HF, and persistent inhibition of sympathetic nerves had adverse effects on the heart.[5] Fukuda, et al[6] reported that the cardiac sympathetic nerves had a complex dynamic remodeling process in HF. Meanwhile, clinical studies found that more than

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30% of chronic HF patients (NYHA Ⅲ or above) had intra-atrial, intra-ventricular or atrioventricular block, accompanying with a prolonged QRS interval (≥120 ms), which increased the hospitalization rate and mortality of HF.[7] Furthermore, research confirmed that an abnormally prolonged QT interval and increased QT dispersion have a positive correlation with sympathetic and para-sympathetic damage.[8,9] Therefore, continuous application of beta-blockers or increasing vagal activity and inhibiting sympathetic activity in decompensated HF, will further aggravate the progress of the disease.

Nerve growth factor (NGF) and leukemia inhibitory factor (LIF) are most important factors in anatomical and functional denervation of sympathetic nerves. In the hypertrophic period of the heart, increased NGF expression gives rise to cardiac sympathetic anatomical hyperinnervation, whereas decreased NGF in end-stage HF produces anatomical denervation.[10,11] During the whole process of HF, rising LIF results in cholinergic transdifferentiation of sympathetic nerves, which is so-called functional deterioration.[12,13] Thus, correcting the balance between NGF and LIF may improve sympathetic innervation in HF.

Guizhi Decoction (桂枝汤) is a representative prescription of Chinese medicine for the treatment of Ying-Wei disharmony. Its therapeutic is thought to be consistent with the pathological mechanism of sympathetic remodeling in the pathological process of HF. Our previous study found that Guizhi Decoction could reverse cardiac sympathetic remodeling induced by streptozotocin (STZ) in diabetic rats by affecting the expression of NGF and ciliary neurotrophic factor (CNTF), which maintains the balance of cardiac sympathetic-vagal.[14] Therefore, we hypothesis that it could correct the imbalance of anatomical and functional innervation of sympathetic nerves by regulating the balance of NGF and LIF. In this study, we observed the effect of Guizhi Decoction on the remodeling of sympathetic and tried to explore its mechanism in decompensated HF.

METHODS

Animals and Treatment

Eighteen specific pathogen free (SPF) grade male Dahl salt-sensitive rats and 9 SS-13^{BN} rats of 6-week-old, weighing 180±20 g, were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. [Beijing, China, animal certificate No. SCXK (Jing) 2016-0011]. The animals were housed 5 per cage in a room maintained at 23–25 ℃ with a 12-h light/dark cycle, food and water were provided *ad libitum*. After the adaptation period, animals were divided into 3 groups : SS-13^{BN}group (n=9), DS group (Dahl salt-sensitive rats, n=9) and GS group (Guizhi Decoction, n=9). Animals were fed with a high-salt (8% NaCl) diet after a week of adaptive feeding. After 10 weeks of a high-salt diet, the GS group rats were given Guizhi Decoction through gastrointestinal administration once a day for 4 weeks, and other two groups were given saline at an equal volume as a vehicle. All procedures were approved by the Faculty of Medicine and Health Sciences Ethics Committee for Animal Research of Shandong University of Traditional Chinese Medicine (Ethics No. SDUTCM2018071501). Every effort was made to minimize pain for the animals.

Drugs

Guizhi Decoction was purchased from the Affiliated Hospital of Shandong University of Traditional Chinese Medicine (Shandong, China) and was composed of *Ramulus Cinnamomi*, *Radix Paeoniae Alba*, and *Radix Glycyrrhizae*. The Chinese herbs were authenticated by Prof. LI Feng in the Pharmacy College, Shandong University of Traditional Chinese Medicine (Shandong, China). *Ramulus Cinnamomi*, *Radix Paeoniae Alba* and *Radix Glycyrrhizae* were mixed in a ratio of 3:2:2 and extracted under reflux with distilled water (1:10 volumes) twice for 1 h each. The extract solution was mixed and concentrated to a relative density of 1.20–1.25 (70–80 ℃). According to a previous method, 1 g granules contain 2 g of raw herbs were prepared to obtain Guizhi Decoction granules.[14] An aqueous solution with 1.5 g crude drug/mL were used for the experiments.

Physiological Measurements

Mean arterial pressure (MAP) of conscious rats were measured from 7:00 am to 12:00 am using tail-cuff blood pressure multi-channel systems (MRBP, IITC Life Science Instruments, USA), every 3 weeks.[15] All rats were measured 3 times to obtain an average value. The body weights of the rats were periodically measured every week.

Echocardiography and Electrocardiogram

Echocardiography and electrocardiogram (ECG) were performed prior to the first and after the

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last gastrointestinal administration, under general anaesthesia with intraperitoneal administration (sodium pentobarbital, 20 mg/kg) as previously described.[16,17] Transthoracic echocardiography were conducted using an M5 Vet Veterinary Ultrasound system (Mindray, Guangdong, China) to assess left ventricular ejection fraction (LVEF) and diastolic thickness of the interventricular septum (IVSd, mm). ECG was recorded at a paper speed of 25 mm/s, and the sensitivity was adjusted so that 1 mV was equivalent to 10 mm deflections.

Tissue and Plasma Collection

Following 28 days of intragastric dosing, each rat was anaesthetized with sodium pentobarbital (40 mg/kg, intraperitoneal injection). The left ventricle of each rat was rapidly collected and was divided into 3 parts. One part was frozen in liquid nitrogen and serially sectioned at a thickness of 4 μm for immunofluorescence assays, another part was fixed in 10% formaldehyde for haematoxylin-eosin (HE) and masson's trichrome (Masson) stainings, and the third part was used for Western blot, quantitative real-time PCR (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA). Whole blood was collected from the abdominal main vein into a plasma separator tube and was centrifuged at 1,000 × *g* for 15 min at 2–8 ℃. The samples were stored at –80 ℃ until use.

HE and Masson Stainings

Morphological changes of myocardium in left ventricle were measured with the HE staining kit (Solarbio, Beijing, China) and the masson trichrome-staining kit (Solarbio).[18] Analysis was performed with ZEN 1.01.0 Imaging analysis software (Carl Zeiss Microscopy GmbH, German).

Sandwich ELISA

Plasma levels of n-terminal pro-brain natriuretic peptide (NT-proBNP) were measured with ELISA kits: NT-proBNP (Cusabio, Cat No. CSB-E08752r, Wuhan, China). The contents of noradrenaline (NA) in plasma and left ventricle were measured using the following ELISA kits: NA (Cusabio, Cat No. CSB-E07022r). All assays were performed according to the manufacturer's instructions. All assay wells were developed with tetramethylbenzidine and measured at 450 nm.

Real-Time Reverse Transcription PCR

Total RNA of the left ventricle sample was extracted using Trizol reagent (Invitrogen, Cat No. 15596-018, USA) following the manufacturer's instructions. The RNA content was assessed and the purity was measured usinga NanoDrop ND-2000 Spectrophotometer (Agilent Technologies, CA, USA). The specific forward/reverse primer sequences (TaKaRa, China) were as follows: NGF: 5'-TGCCAAGGACGCAGCTTTC-3'/5'-TGAAGTTTAGTCCAGTGGGCTTCAG-3'; LIF: 5'-ATCAAGAGTCAACTGGCTCAACTCA-3'/5'-TGTTGGGCGCACATAGCTTATC-3'; GAPDH: 5'-ATGACCCCTTCATTGACCTCA-3'/5'-GAGATGATGACCCTTTTGGCT-3'. A Prime Script RT reagent kit with gDNA Eraser was used (Takara BioInc, Code No. RR047A, Japan) for reverse transcription of the DNA. Real-time PCR was performed with a LightCycler 480SYBRPremix Ex Taq Ⅱ (Roche, Mannheim, German). Each RNA sample was tested in triplicate, and the threshold cycle values were normalized to GAPDH. The level of expression for each gene was calculated with the $2^{-\triangle\triangle CT}$method.[19]

Western blot Analysis

Frozen left ventricular was homogenized in a RIPA lysis buffer, and the protease inhibitor PMSF was added. The homogenate was incubated for 30 min at 4 ℃ and centrifuged at 15,000 × *g* for 20 min at 4 ℃. The total protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit. Extracts (40 μg) were subjected to electrophoretic separation through a 10%–12% dodecyl sulfate, sodium salt (SDS)-polyacrylamide gel electrophoresis and subsequently transferred to a polyvinylidene fluoride (PVDF) membrane. The PVDF membranes were incubated with the primary antibody and then incubated with corresponding secondary antibodies. The above reagents were purchased from Beyotime Bitechnology, Shanghai, China. The following antibodies were used: NGF [EP1320Y] (Abcam, Cat No. ab52918, Shanghai, China), LIF (Abcam, Cat No. ab113262), tyrosine hydroxylase [EP1532Y] (TH, Abcam, Cat No. ab137869), choline acetyltransferase [EPR16590] (CHAT) (Abcam, Cat No. ab178850), GAP43 (Abcam, Cat No. ab16053), and β-actin (Proteintech, Cat No. 20536-1-AP, Wuhan, China). The corresponding secondary antibody peroxidase-conjugated goat anti-rabbit IgG (ZSGB-Bio, Cat No. ZB2301, Beijing, China) was used. The bands corresponding to the proteins of interest were scanned and band density was analysed using FluorChem Q

3.4. (ProteinSimple, USA). All quantitative analyses were normalized to β-actin.

Immunofluorescence Staining in the Left Ventricle

Sample fixation, embedding, sectioning, and blocking were conducted using standard procedures.[20] Noradrenergic nerve fibres were stained using rabbit anti-rat TH (Abcam, ab112, 1:100), cholinergic cell bodies and nerve fibres were stained using goat anti-rat CHAT (Novus, NBP1-30052, 1:100, USA), and neuronal growth cones were stained using mouse anti-rat GAP43 (Abcam, ab129990, 1:100). The following secondary antibodies were used: donkey anti-rabbit IgG H&L (Alexa Fluor® 647) (Abcam, ab150075, 1:200, Shanghai, China), donkey anti-goat IgG H&L (FITC) (Abcam, ab6881, 1:500), and goat anti-mouse IgG H&L (FITC) (Abcam, ab6785, 1:600). The stained slides were observed under a Zeiss Vert A1 fluorescence microscope (Carl Zeiss Jena, German).

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 software (SPSS, USA). The values are presented as the mean ± standard deviation ($\bar{x} \pm s$). Data were analyzed by one-way analysis of variance (ANOVA), followed by Student's *t*-tests. Differences between groups were considered statistically significant when the two-sided $P<0.05$.

RESULTS

Blood Pressure and Body Weight

MAP in the DS group was elevated and significantly higher compared to the SS-13^{BN} group after 6 weeks ($P<0.01$). The blood pressure of the DS and GS groups reached the highest value at 10 weeks, but there was no difference between the two groups. In contrast, after the treatment of Guizhi Decoction, blood pressure was significantly reduced compared to the DS group at 14 weeks ($P<0.05$, Figure 1A). After 14 weeks, the body weights of rats in the SS-13^{BN} group were significantly increased compared to the hypertensive group ($P<0.01$). However, the body weights of the GS group showed a slow downward trend, which was significantly larger compared to the DS group at 14 weeks ($P<0.05$, Figure 1B).

NT-proBNP, Echocardiography and ECG

As shown in Figure 2A, NT-proBNP was significantly decreased in the GS group compared with

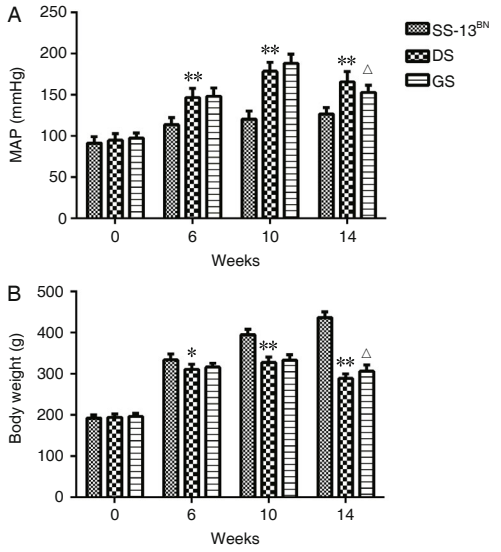

Figure 1. Blood Pressure (A) and Body Weight (B) Measured from Different Groups (n=9, $\bar{x} \pm s$)

Notes: $^{*}P<0.05$, $^{**}P<0.01$ *vs*. SS-13^{BN} group; $^{\triangle}P<0.05$, *vs*. DS group

the DS group ($P<0.01$). The IVSd of DS group was significantly higher compared with the SS-13^{BN} group at 10 weeks of age ($P<0.01$). Treatment of Guizhi Decoction reduced IVSd compared with the DS group at 14 weeks, but there were no differences between the two groups (Figure 2B). LVEF was decreased in the GS group and showed a significant slow downward trend compared with that in the DS group at 14 weeks ($P<0.01$, Figure 2C). The rats in the DS group showed a prolonged P-R interval and slowed heart rate in the Ⅰ–Ⅲ leads compared with the SS-13^{BN} group at 14 weeks (Figures 2D and 2E). Guizhi Decoction reduced the P-R interval and increased the heart rate compared to the DS group (Figure 2F).

Histopathology

As shown in Figure 3A, DS rats exhibited more cardiomyocyte degeneration and focal inflammatory cell infiltration than the SS-13^{BN} group. Guizhi Decoction administration significantly decreased the inflammatory infiltration compared with the DS group. As shown in Figure 3B. Myocardial fibrosis and collagen deposition were decreased in the GS group compared to the DS group.

NA in the Plasma and Left Ventricle

Compared with SS-13^{BN} group, plasma NA level increased and myocardial NA level decreased in DS group. Guizhi Decoction significantly increased the content of myocardial NA and reduced the plasma NA level compared with the DS group ($P<0.05$ or $P<0.01$,

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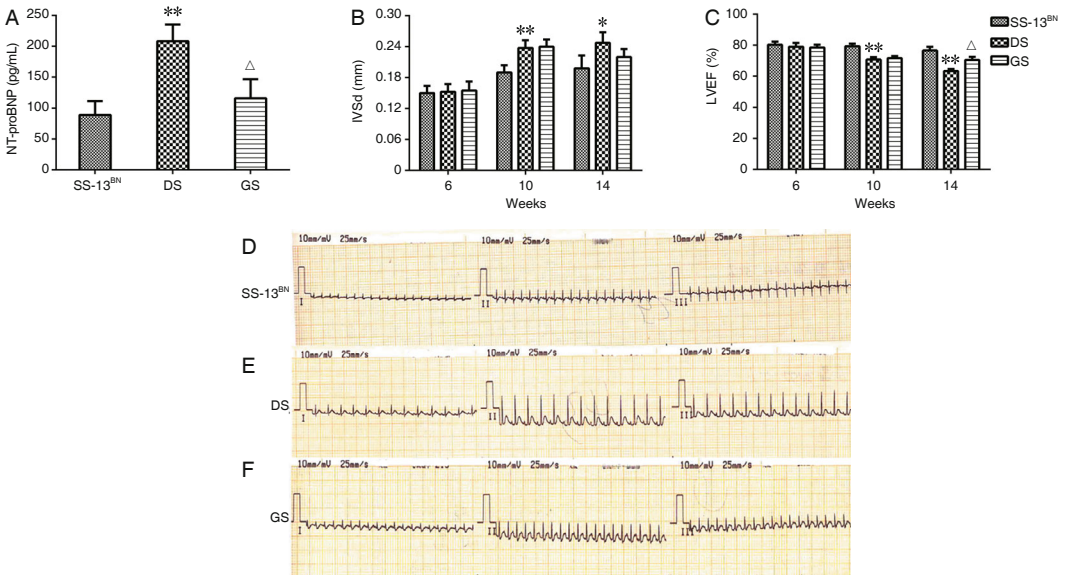

Figure 2. Changes in NT-proBNP (A), Echocardiographic (B-C) and ECG (D-F) in Different Groups (n=6, $\bar{x} \pm s$)

Notes: $^{*}P$<0.05, $^{**}P$<0.01 *vs*. SS-13^{BN} group; $^{\triangle}P$<0.01 *vs*. DS group

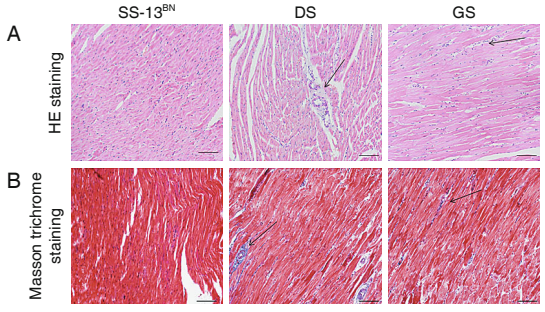

Figure 3. Myocardial Morphology of Left Ventricle by HE (A) and Masson Stainings (B)

Notes: The black arrow indicates the location of the lesion, n=4, scale bars: 50 μm

Figures 4A and 4B).

Gene and Protein Expression of NGF and LIF

As shown in Figures 5A–5F, the NGF mRNA and protein expression decreased, and those of LIF increased in the DS group compared with the SS-13^{BN} group. After 4 weeks of administration, Guizhi Decoction significantly increased the level of NGF mRNA and protein expression while those of LIF were reduced compared with the DS group (P<0.05 or P<0.01).

Differential Expression of Cardiac Sympathetic and Parasympathetic Nerves

According to the double immunofluorescence staining and Western blot analysis results, compared to the SS-13^{BN} group, the desity of TH^{+}/GAP43^{+} positive nerve fibres and GAP43 protein levels were decreased in the DS group. In DS group, few of coexpression of GAP43^{+} positive nerve fibre areas were observed

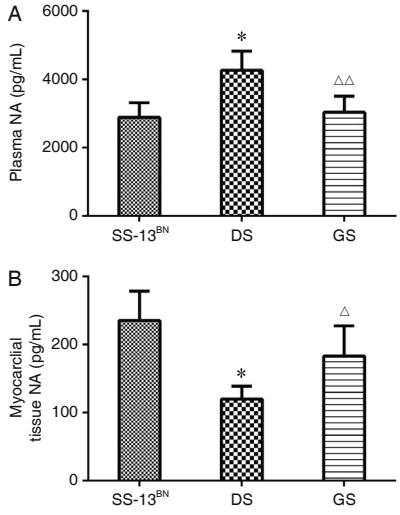

Figure 4. Detection of NA Concentrations in Plasma (A) and Left Ventricle (B) by ELISA (n=6, $\bar{x} \pm s$)

Notes: $^{*}P$<0.01 *vs*. SS-13^{BN} group; $^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01 *vs*. DS group

in TH^{+}. Meanwhile, the desity of TH^{+}/CHAT^{+} positive nerve fibres and TH protein levels were decreased in the DS group, while the expression of CHAT had no significant changes. After 4 weeks of administration, Guizhi Decoction significantly increased the expression of TH^{+}/GAP43^{+} and TH^{+}/CHAT^{+} positive nerve fibres, and the protein levels of GAP43 and TH were similarly raised compared with the DS group (P<0.01, Figure 6 and Figure 7).

DISCUSSION

NGF is a prototypic member of the neurotrophin

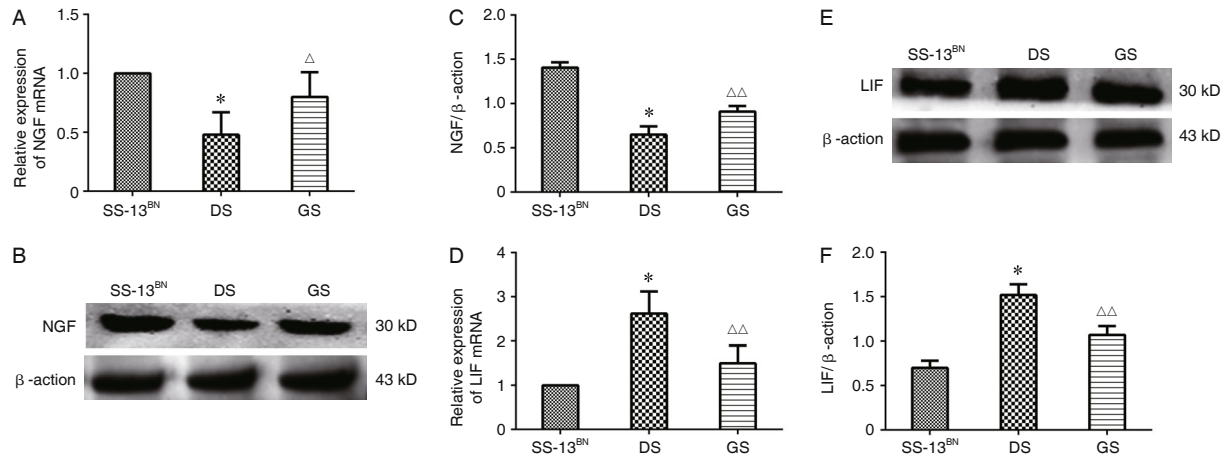

Figure 5. Expression of Myocardial NGF (A–C) and LIF (D–F) Measured by qRT-PCR or Western Blot (n=4, $\bar{x} \pm s$)

Notes: *P<0.01 *vs*. SS-13^{BN} group; $^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01 *vs*. DS group

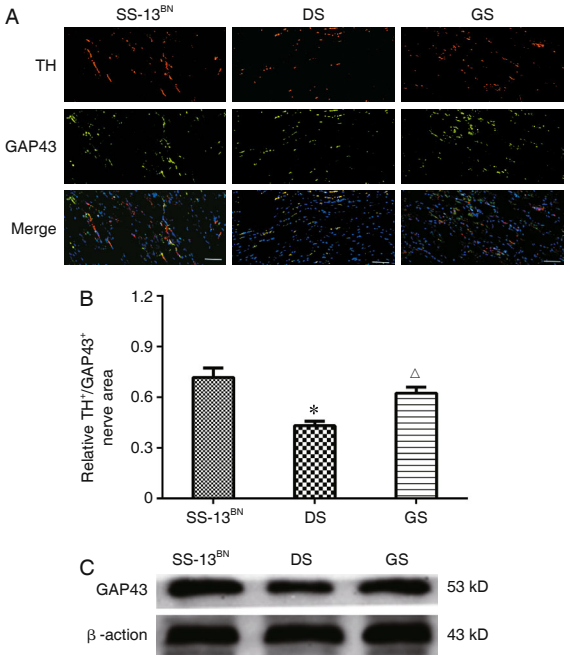

Figure 6. Anatomical Denervation of Sympathetic Nerves (A-C) Determined by Double Immunofluorescence or Western Blot (n=3, $\bar{x} \pm s$)

Notes: *P<0.01 *vs*. SS-13^{BN} group; $^{\triangle}P$<0.01 *vs*. DS group; scale bars: 50 μm

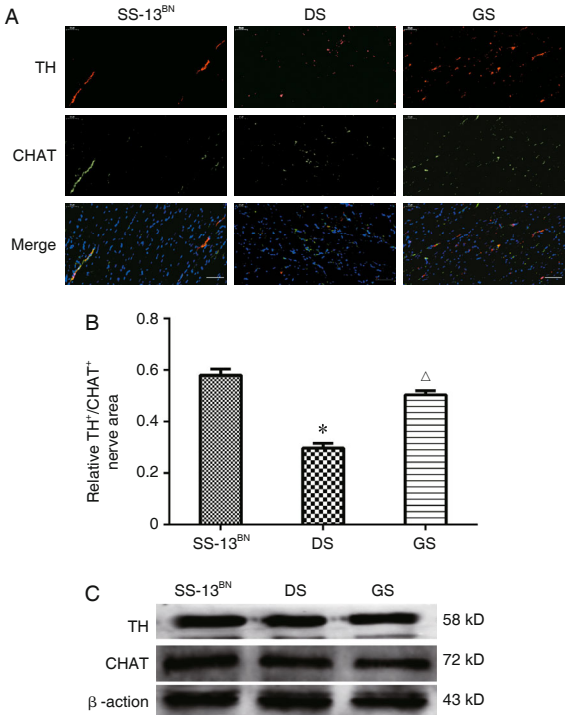

Figure 7. Cholinergic Transdifferentiation of Sympathetic Nerves (A-C) Determined by Double Immunofluorescence or Western Blot (n=3, $\bar{x} \pm s$)

Notes: *P<0.01 *vs*. SS-13^{BN} group; $^{\triangle}P$<0.01 *vs*. DS group; scale bars: 50 μm

family, which is widely recognized as a family of essential factors for synaptic activity in peripheral sympathetic systems.[21] It has been reported that NGF expression within myocardial tissue corresponds to the levels of sympathetic innervation density,[22] and NGF plays a crucial role in sympathetic nerves sprouting, which leads to the regeneration of cardiac sympathetic nerves and abnormal innervation in failing hearts.[23] GAP43 is a specific protein involved in nerve axon growth, which is tightly associated with neuronal development and axonal regeneration.[24]

The overexpression of GAP43 leads to the anatomical hyperinnervation of sympathetic nerves in injured myocardium, which can be used to investigate whether the reduction of NGF in salt-sensitive hypertension rats could affect nerve innervation density in the left ventricle.[24,25] Our study found that the decrease of NGF mRNA/protein expression resulted in the down-regulation of GAP43 immunofluorescence/protein expression and induced the anatomical denervation

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of sympathetic nerves. Meanwhile, we observed that decreased level of NGF was concomitanted with the high level of NA concentration in plasma. As a neurotransmitter of sympathetic nerve, the decreased level of NA in myocardial tissues indicates the decrease level of TH in failing hearts. Higher plasma NA concentration is due to the increased release of NA in myocardium and the impairment of NA re-uptake and synthesis.[25-27] However, there is no clear evidence showing higher concentration of plasma norepinephrine (NE) directly affects apoptosis of sympathetic neurons, but it has been confirmed that long-exposure of plasma NE contributes to the NGF consumption in failing hearts, which is a secondary effect on the attenuation of cardiac sympathetic fibres.[28]

The molecular mechanism of how plasma NE induces NGF down-regulation is not yet clear. Kaye, et al[29] reported that in an experimental model of chronic HF, excessive release of plasma NE depressed expression of NGF and its neurotrophic receptor tyrosine kinase A (TrKA), which reduced the density of sympathetic neurons. Qin, et al[30] reported that the decrease of cardiac sympathetic transmitters was closely related to the reduction of NGF and TrKA mediated by plasma NE. However, injecting NGF into the stellate ganglia improved the NA uptake in myocardium, which indicated that diminished NGF limits cardiac regenerative in failing hearts. The mortality rate of HF model was reduced after addition of NGF.[31,32] Thus, NGF serves as a positive regulator of NE re-uptake and it is feasible to improve myocardial NE uptake and restore innervation of anatomical sympathetic nerves by regulating NGF in treating HF.[31]

LIF is a member of the interleukin-6 cytokine family,[33] which regulates the growth and differentiation of many cell types and participates in inflammation, neurodevelopment, haematopoiesis, and embryogenesis, etc.[34-36] LIF transmits signals through gp130 receptors and overlaps with other family members that have unique biological functions.[37,38] In terms of neurogenesis, LIF acts as a sympathetic switch to promote the differentiation of sensory and motor neurons and as well as glial cells.[38] Studies have shown that LIF could cause cholinergic transdifferentiation of cardiac sympathetic nerves via gp130-signalling cytokines and lead to sympathetic dysfunction in HF.[39] In our study, with the increase of plasma NT-proBNP and the decrease of LVEF, myocardial injury was aggravated in rats, which further stimulated the expression of LIF mRNA/protein. The overexpresion of LIF induced the down-regulation of TH immunofluorescence/protein expression. According to the constant level of CHAT, decreased level of TH led to more serious imbalance of sympathetic/para-sympathetic. These results suggested the elevation of myocardial LIF promoted cholinergic transdifferentiation and denervation of sympathetic function during severe HF.

However, the pros and cons of cholinergic transdifferentiation are not clear. One possibility is that it is a side effect, and failing hearts may require up-regulation of LIF, which may accidentally lead to transdifferentiation and sympathetic denervation. Another possibility is that cholinergic transdifferentiation may be an adaptive response to protect the heart.[39] Our results suggest that cholinergic transformation leads to a prolonged P-R interval in ECG and as well as myocardial pathological injury. After using the Guizhi Decoction, we found that it not only inhibited cholinergic transdifferentiation and increased sympathetic innervation, but also improved myocardial injury and cardiac function. Therefore, our study suggested that the cholinergic transdifferentiation may be a disadvantageous outcome in severe HF. This speculation is strongly supported by the study of Fernandez, et al. They reported that the up-regulation of cardiomyocyte muscarinic receptors is a double-edged sword in failing hearts, and the increase in cholinergic stimulation adversely attenuates cardiac contractility and depresses cardiac function; in contrast, restoring the normal neurotransmitter composition of sympathetic nerves may become important new targets to treat advanced HF.[40]

Studies have demonstrated that sympathetic neurotransmitter conversion or cholinergic transdifferentiation may also occur in the pathophysiological events of adult chronic HF.[41,42] This view has been confirmed by Kanazawa, and the TH^+ nerves of the epicardial nerve bundle were predominant in the control hearts, whereas the $CHAT^+$ nerves were predominant in severe failing hearts and immunofluorescence staining of stellate ganglion confirmed that sympathetic neurotransmitters in chronic HF patients could be converted from catecholaminergic to cholinergic.[39] Currently, the significance of cardiac

sympathetic cholinergic transdifferentiation needs further clarification. Our study suggested that cholinergic transdifferentiation was a disadvantageous factor because the heart rate of rats decreased, the P-R interval was prolonged, cardiac function decreased, and myocardial pathological injury was aggravated in the decompensation period of HF. Several studies have proved that enhancement of vagal tone prolonged the effective refractory period and slowed the concealed conduction in the AV node.[43,44] Meanwhile the use of β-receptor blockers could exacerbate bradycardia and atrioventricular block as well as increase mortality. Thus, inhibiting the cholinergic transdifferentiation of sympathetic nerves and increasing the dominance of sympathetic nerves in the decompensated period of HF may be a new direction for future treatments.

In this study, we showed that high-salt intake led to cardiac function impairment and anatomical and functional imbalance of sympathetic nerves in DS rats. We demonstrated that treatment with Guizhi Decoction inhibited myocardial interstitial fibrosis, attenuated inflammatory cells infiltration, prevented the cholinergic transdifferentiation, promoted sympathetic rejuvenation, restored the balance of anatomical and functional innervation of sympathetic nerves and delayed the progress of HF. The mechanism may be related to correcting the balance of NGF and LIF.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

Wang YC performed experiments, data analysis and drafted the manuscript. Ma DF and Jiang P carried out data collection and analysis. Zhou GF, Li ZY, Zhang YM and Yang JL were responsible for animal care and telemetry. Li X designed the study, supervised the experiment and revision of the manuscript. All authors read and approved the final manuscript.

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