Chin J Integr Med 2014 Jul;20(7):524-533

ORIGINAL ARTICLE

Effect of Guizhi Decoction (桂枝汤) on Heart Rate Variability and Regulation of Cardiac Autonomic Nervous Imbalance in Diabetes Mellitus Rats*

LI Xiao (李　晓)[1], JIANG Yue-hua (姜月华)[1], JIANG Ping (姜　萍)[2], YANG Jin-long (杨金龙)[2], MA Du-fang (马度芳)[2], and YANG Chuan-hua (杨传华)[1]

ABSTRACT **Objective**: To observe abnormalities in heart rate variability (HRV) in diabetic rats and to explore the effects of treatment with Guizhi Decoction (桂枝汤) on cardiac autonomic nervous (CAN) imbalance. **Methods**: A radio-telemetry system for monitoring physiological parameters was implanted into rats to record electrocardiac signals and all indictors of HRV [time domain measures: standard deviation of all RR intervals in 24 h (SDNN), root mean square of successive differences (RMSSD), percentage of differences between adjacent RR intervals greater than 50 ms (PNN50), and standard deviation of the averages of RR intervals (SDANN); frequency domain measures: low frequency (LF), high frequency (HF), total power (TP), and LF/HF ratio]. The normal group was randomly selected, and the remaining rats were used to establish streptozocin (STZ)-induced diabetic model. After 4 weeks, the model rats were divided into the model group, the methycobal group, and the Guizhi Decoction group, 9 rats in each group. Four weeks after intragastric administration of the corresponding drugs, the right atria of the rats were collected for immunohistochemical staining of tyrosine hydroxylase (TH) and choline acetyltransferase (CHAT) to observe the distribution of the sympathetic and vagus nerves in the right atrium. The myocardial homogenate from the interventricular septum and the left ventricle was used for determination of TH, CHAT, growth-associated protein 43 (GAP-43), nerve growth factor (NGF), and ciliary neurotrophic factor (CNTF) levels using an enzyme-linked immunosorbent assay. **Results**: (1) STZ rats had elevated blood glucose levels, reduced body weight, and decreased heart rate; there was no difference between the model group and the drug treated groups. (2) Compared with the model group, only RMSSD and TP increased in the methycobal group significantly ($P<0.05$); SDNN, RMSSD, PNN50, LF, HF, and TP increased, LF/HF decreased ($P<0.05$), and SDANN just showed a decreasing trend in the Guizhi Decoction group ($P>0.05$). TH increased, CHAT decreased, and TH/CHAT increased in the myocardial homogenate of the model group ($P<0.05$). Compared with the model group, left ventricular TH reduced in the methycobal group; and in the Guizhi Decoction group CHAT increased, while TH and TH/CHAT decreased ($P<0.05$). Compared with the model group, CNTF in the interventricular septum increased in the methycobal group ($P<0.05$); GAP-43 increased, NGF decreased, and CNTF increased ($P<0.05$) in the Guizhi Decoction group. There were significant differences in the reduction of NGF and elevation of CNTF between the Guizhi Decoction group and the methycobal group ($P<0.05$). (3) Immunohistochemical results showed that TH expression significantly increased and CHAT expression significantly decreased in the myocardia of the model group, whereas TH expression decreased and CHAT expression increased in the Guizhi Decoction group ($P<0.05$). **Conclusion**: Guizhi Decoction was effective in improving the function of the vagus nerve, and it could alleviate autonomic nerve damage.

KEYWORDS diabetes mellitus, cardiac autonomic neuropathy, sympathetic-vagal imbalance, heart rate variability, Chinese medicine, Guizhi Decoction

Cardiac autonomic nerves (CANs) include the sympathetic nerve and the vagus nerve, which check and balance each other and play important roles in the regulation of heart rate, conduction, and cardiac contractility. Diabetic cardiac autonomic neuropathy is one of the severe complications of

*Supported by the National Natural Science Foundation of China (No. 81072962) and the Special Fund of Taishan Scholars Program of Shandong Province (No. 2012-55)

1. Department of Cardiology, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan (250011), China; 2. Shandong University of Traditional Chinese Medicine, Jinan (250355), China

Correspondence to: Prof. YANG Chuan-hua, Tel: 86-531-68616008, E-mail: yangchuanhua1962@126.com

DOI: 10.1007/s11655-014-1861-z

diabetes mellitus; damage of CANs in a high glucose environment causes sympathetic-vagal imbalance,[1,2] which thus causes abnormal neurotransmitter signaling and increased asynchronization of cardiac electrophysiology.[3,4] The structure and damage repair capacity of the sympathetic and vagus nerves are different, which exacerbates autonomic nervous system imbalance and greatly increases the incidence of diabetic cardiomyopathy, arrhythmia, painless myocardial ischemia, and sudden death.

According to a research, strict blood sugar control could slow the progression of CAN diseases but could not reverse CAN pathological changes.[5] Therefore, in addition to protecting the sympathetic and vagus nerves from further damage, regulation of the imbalance of these two nerves is even more important for the treatment of diabetic CAN diseases. These views of harmony and adjustment are very much in line with the theory of Ying (营, nutrient) and Wei (卫, defense) and the treatment method and principle of harmonizing Ying and Wei in Chinese medicine (CM). The representative prescription formula is Guizhi Decoction (桂枝汤).

Guizhi Decoction is a classic prescription with unique characteristics in CM. Our previous studies have shown that based on the treatment methods of balancing yin and yang and harmonizing Ying and Wei in CM theory, the administration of Xinhe Granules (心和颗粒) and Tiaoxin Decoction (调心饮), which contain the components of Guizhi Decoction, could improve symptoms of autonomic neuropathy in coronary heart disease.[6,7] Studies also confirmed that Guizhi Decoction decreased the accumulation of inflammatory factors including nuclear factor κB (NF-κB), interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and endothelin (ET-1) in the heart of streptozocin (STZ) rats; reversed spontaneous myocardial collagen remodeling in diabetic rats; significantly reduced myocardial basement membrane thickness; prevented damage and thickening of the myocardial basement membrane; and improved oxygen diffusion barriers,[8,9] thus to prevent and treat diabetic myocardial damage. Some studies showed that methycobal protected autonomic nerves; in comparison, we investigated whether Guizhi Decoction was a better treatment and balance adjustment option for the imbalance of CAN damage caused by diabetes mellitus.

Heart rate variability (HRV) is the phenomenon of variation in the time interval between sinus heart rates, which reflects the tone between the sympathetic and parasympathetic nerves and their balance. It is a sensitive quantitative indicator for monitoring autonomic neuropathy and imbalance. To avoid the effect of animal restraint and anesthesia on HRV, this study employed telemetry to record 24 h electrocardiac signals when animals were unrestrained, which accurately evaluates the balance between sympathetic and parasympathetic nerves. On this basis, we further measured the concentration and expression of tyrosine hydroxylase (TH) and choline acetyltransferase (CHAT) and detected a relevant neurotrophic factor that may reflect nerve damage and repair to confirm and explore the effects of Guizhi Decoction on the regulation of the imbalance of autonomic nerve damage.

METHODS

Animals

Thirty-six specific pathogen free (SPF) grade male Wistar rats of 8-week-old, weighing 280–300 g, were provided by Jining Lukang Experimental Animal Center [Permit No. SCXK (Lu) 20080002].

Reagents and Instruments

STZ was from Sigma-Aldrich (USA, lot: 329A039). Mecobalamin tablets (methycobal) were from Eisai China, Inc. (lot: 120307A). The TH enzyme-linked immunosorbent assay (ELISA) kit, CHAT ELISA kit, nerve growth factor (NGF) ELISA kit, and growth-associated protein 43 (GAP-43) ELISA kit were products of Bioyun Inc., Shanghai, China (lots: 20120704, 20120704, 20120728, and 20120704, respectively). The ciliary neurotrophic factor (CNTF) ELISA kit was from PeproTech Inc., Rocky Hill, NJ, USA (lot: 0711065). Rabbit anti-rat TH polyclonal IgG, rabbit anti-rat CHAT polyclonal IgG, goat anti-rabbit secondary IgG, the streptavidin-biotin complex (SABC) reagent kit, 5% bovine serum albumin (BSA), and the diaminobenzidine chromogenic kit were from Wuhan Boster Biological Technology., Ltd., China (lots: 11S47, 38244, 08G23C, 08G24C, 08E31C, and 08D18B22, respectively).

The radio-telemetry system for monitoring physiological parameters (implantable ECG telemetry system) was from Data Sciences International (DSI, St. Paul, Minnesota, USA). The T25 homogenizer was from IKA (Germany). The ELx808 microplate reader was from BIO-TEK (USA). The MR23i refrigerated centrifuge was from Jouan (France). The HPIAS-1000 high definition color pathological image analysis

system was from Champion Images of Tongji Medical University (Wuhan, China).

Experimental Drugs

The Chinese herbals used in the study were identified by Prof. FENG Li in the Pharmacy College, Shandong University of Traditional Chinese Medicine. According to ancient prescriptions, raw material of *Cassia twig*, *Paeoniae Radix Alba* and *Glycyrrhizae uralensis* were kept at the ratio of 3:3:2, and reflux extraction was performed twice using 10 volumes of water; the first extraction was decocted for 1.5 h, and the second was for 1 h. The extracts were combined, filtered, and concentrated under reduced pressure to a relative density of 1.20–1.25 (70–80 ℃). Excipient of powdered erythrose alcoholand stevioside (99:1) granules were then added and dried below 60 ℃ to obtain Guizhi Decoction granules at 2 g granules/g raw herbs. For usage, it was prepared as an aqueous solution at 1 g raw medicinal herbs/mL solution. Drugs such as methycobal were prepared as suitable suspensions.

Recording and Analysis of HRV

This study used telemetry technology to record electrocardiac signals and to calculate all indicators of HRV when animals were untreated and unrestrained to avoid the effect of animal restraint and anesthesia on HRV. The linear analyses of HRV included time domain analysis and frequency domain analysis.

The physiological parameter telemetry system was used for recording HRV. The implanter was surgically implanted into the abdominal cavity of rats. Before surgery, the implanter was disinfected, and the abdominal skin was cut open. The placement of ECG electrodes followed the limb lead Ⅱ form. The electrode lead and implanter were placed under the skin and were fixed with sutures. After surgery, intramuscular injection of penicillin was performed for 3 consecutive days to prevent infection.

On the 8th day after surgery, a 24-h ECG was recorded and analyzed. The Kubios HRV_2.0 software (University of Kuopio, Finland) was used to analyze and measure all HRV indicators to use as the HRV of normal rats. The HRV indicators mainly included the time domain indicators standard deviation of all RR intervals in 24 h (SDNN), standard deviation of averages of RR intervals (SDANN), room mean square of successive differences (RMSSD), and percentage of differences between adjacent RR intervals greater than 50 ms (PNN50) and the frequency domain indicators: very low frequency (VLF), low frequency (LF), high frequency (HF), and LF/HF ratio.

Grouping and Establishment of Diabetes Rat Model

Nine rats were randomly selected as the normal group, and the other 27 rats were as the modeling group.

STZ was dissolved in sodium citrate buffer solution (pH 4.4) and was intraperitoneally injected into rats at 60 mg/kg. The rats in the normal group received an equal amount of sodium citrate buffer solution. The one touch profile blood glucose meter by Johnson & Johnson was used to measure fasting blood glucose every day. Blood glucose was stabilized and maintained at >16.7 mmol/L by subcutaneous injection of insulin or supplemented with intraperitoneal injection of STZ (10 mg/kg). After the model was successfully established, body weight, blood glucose, and heart rate were measured each week. The model was maintained for 4 weeks, and HRV was measured each week. The SDNN indicator was obtained to ensure that there was no difference among the groups. The modeling animals were randomly divided into 3 groups, the model group, the methycobal group, and the Guizhi Decoction group, 9 rats in each group.

Administration of Drugs

The drug dose was equivalent to 10 times the amount orally administered for humans. Four weeks after establishment of the model, rats in the blank control and the model groups received intragastric administration with normal saline 2 mL, those in the methycobal and the Guizhi Decoction groups with methycobal 2 mL [0.15 mg/(kg·day)] or Guizhi Decoction 2 mL [4.0 g crude drug/(kg·day)], respectively, and the administration course was 4 weeks; HRV was measured every week. All the rats were fed with normal diet during drug administration.

Collection of Heart Tissues

Four weeks after intragastric administration, the rats were fasted overnight, and fasting blood glucose was measured the next day. The rats were anesthetized by intraperitoneal injection of 4% sodium pentobarbital (40 mg/kg), and the implanter was removed. The heart was removed by thoracotomy, and the following procedure was performed on ice.

At the upper 1/3 position behind the heart (right and left atrium), the entire cross-section of the heart were taken at an angle of 30°. At this angle, the left and right atrium and ventricle could be displayed together. Right atrial tissues at a thickness of 0.5 cm were trimmed, collected, fixed in neutral formalin, dehydrated, fixed, embedded, and serially sectioned. Each section had a thickness of 4 μm and was used for subsequent detection. The interventricular septum and left ventricle were collected and then shredded using tissue scissors; this homogenate was diluted 5-fold with normal saline and centrifuged at 4,000 r/min for 10 min to prepare a 20% myocardial tissue homogenate. The supernatant was stored at –20 ℃ for subsequent measurement.

Quantitative Measurement of TH, CHAT, NGF, and CNTF in Myocardia

The interventricular septum and left ventricle homogenate were used for TH, CHAT, GAP-43, NGF, and CNTF measurement using ELISA kits strictly according to the instruction manuals of the kits. The absorbance at 450 nm was measured using a microplate reader. The concentrations of TH, CHAT, GAP-43, NGF, and CNTF in myocardia were calculated according to the standard curve.

Immunohistochemical Staining of TH and CHAT in the Right Atrium

For the SABC method, paraffin sections were conventionally deparaffinized to water. Endogenous peroxidases were eliminated by incubating with 3% H_2O_2 at room temperature; after washing with phosphate buffer solution (PBS) 3 times for 5 min, antigen retrieval was performed by microwaving. Sections were blocked with 5% BSA at room temperature for 30 min and then incubated with rabbit anti-rat TH or CHAT primary antibodies (1:100 dilution) in a moisture box at 4 ℃ overnight. After washing with PBS 3 times for 5 min, sections were stained with goat anti-rabbit IgG secondary antibodies and incubated at room temperature for 60 min. After washing with PBS 3 times for 5 min, SABC was added, incubated at room temperature for 30 min and again washed with PBS 3 times for 5 min. Color was developed using DAB, and the development was controlled under a microscope. Sections were washed with distilled water, counterstained with hematoxylin, dehydrated, cleared, and mounted using neutral balsam. A rat brain section was used as a positive control, and a section where the primary antibody was replaced by PBS was used as a negative control. The sites with brown-yellow granule precipitation were judged as positive. Each section was observed under a light microscope with a 20× objective lens; images of 5–10 fields were collected and transmitted to the HPIAS-1000 high definition color pathological image analysis system. Under the same background and magnification (20× magnification, pixel length: 0.816 mm), brown-yellow color was selected as a positive surface density of TH or CHAT, and the other areas were used as a negative surface density. The percentage positive surface density of all cells was calculated. The calculation formula was as follows: TH (or CHAT) positive surface density ratio=TH (or CHAT) positive surface density/(positive surface density + negative surface density). This was used as an indicator to evaluate the expression of TH (or CHAT).

Statistical Analysis

The statistical analysis of data was performed using the SPSS 17.0 software. The measurement data were presented as mean ± standard deviation. Comparison between groups was performed using single-factor analysis of variance (ANOVA).

RESULTS

General Condition of Animals

After intraperitoneal injection of STZ, rats had increased eating and drinking, frequent urination, weight loss, and poor mental state. The successful establishment of the diabetes mellitus model was determined by a fasting blood glucose ⩾11.1 mmol/L or a random blood glucose ⩾16.7 mmol/L. After the factors of failed modeling and death were excluded, each group contained 6 animals. During the experimental process, the body weight of rats in the normal group rose steadily at a rate that was significantly higher than in the modeled groups. The model was successfully established, the diabetic rats had decreased body weight, elevated blood glucose levels, and decreased heart rate; there was no difference among the three experimental groups (Table 1).

Changes in HRV

Analysis of dynamic changes in time domain indicators showed that after STZ injection, the HRV of the rats significantly changed, in the time domain indicators of the model group, SDNN, RMSSD and PNN50 decreased (P<0.05), and SDANN increased (P<0.05). Methycobal increased the level of RMSSD (P<0.05). In the Guizhi Decoction group, SDNN, RMSSD, and PNN50 increased (P<0.05), and SDANN

Chin J Integr Med 2014 Jul;20(7):524-533

Table 1. Changes of Body Weight, Blood Glucose, and Heart Rate in Rats after Modeling ($\bar{x} \pm s$)

Group	Time	Body weight (g)	Blood glucose (mmol/L)	Heart rate (beat/min)
Normal	Before modeling	320.3 ± 8.4	5.45 ± 0.75	357.6 ± 25.6
	After modeling	453.0 ± 18.7	6.07 ± 0.61	367.2 ± 28.8
DM	Before modeling	324.2 ± 14.5	5.48 ± 0.87	360.6 ± 32.9
	After modeling	315.3 ± 8.3*	22.03 ± 3.45*	293.1 ± 31.4*
Methycobal	Before modeling	319.5 ± 13.3	5.91 ± 1.05	367.3 ± 33.7
	After modeling	303.4 ± 9.4*	21.45 ± 1.62*	284.1 ± 28.0*
Guizhi Decoction	Before modeling	317.0 ± 9.5	5.32 ± 0.84	377.7 ± 32.3
	After modeling	298.2 ± 9.7*	22.51 ± 2.36*	291.9 ± 28.5*

Note: *P<0.05, compared with the normal group

showed a decreasing trend (P>0.05, Figure 1).

LF, HF, and TP decreased (P<0.05), whereas LF/HF increased (P<0.05) in diabetic rats. Compared with the model group, the rats in the methycobal group had a significant increase in TP (P<0.05). Guizhi Decoction increased LF, HF and TP, and decreased LF/HF of the rats (P<0.05, Figure 1).

Changes in the Concentrations of TH and CHAT in the Interventricular Septum and Left Ventricle

This study measured the concentration and distribution of TH and CHAT to determine the function and distribution of sympathetic and parasympathetic nerves. As shown in Figure 2, in the myocardial homogenate of the model group, TH increased, CHAT decreased, and TH/CHAT increased (P<0.05). Methycobal reduced

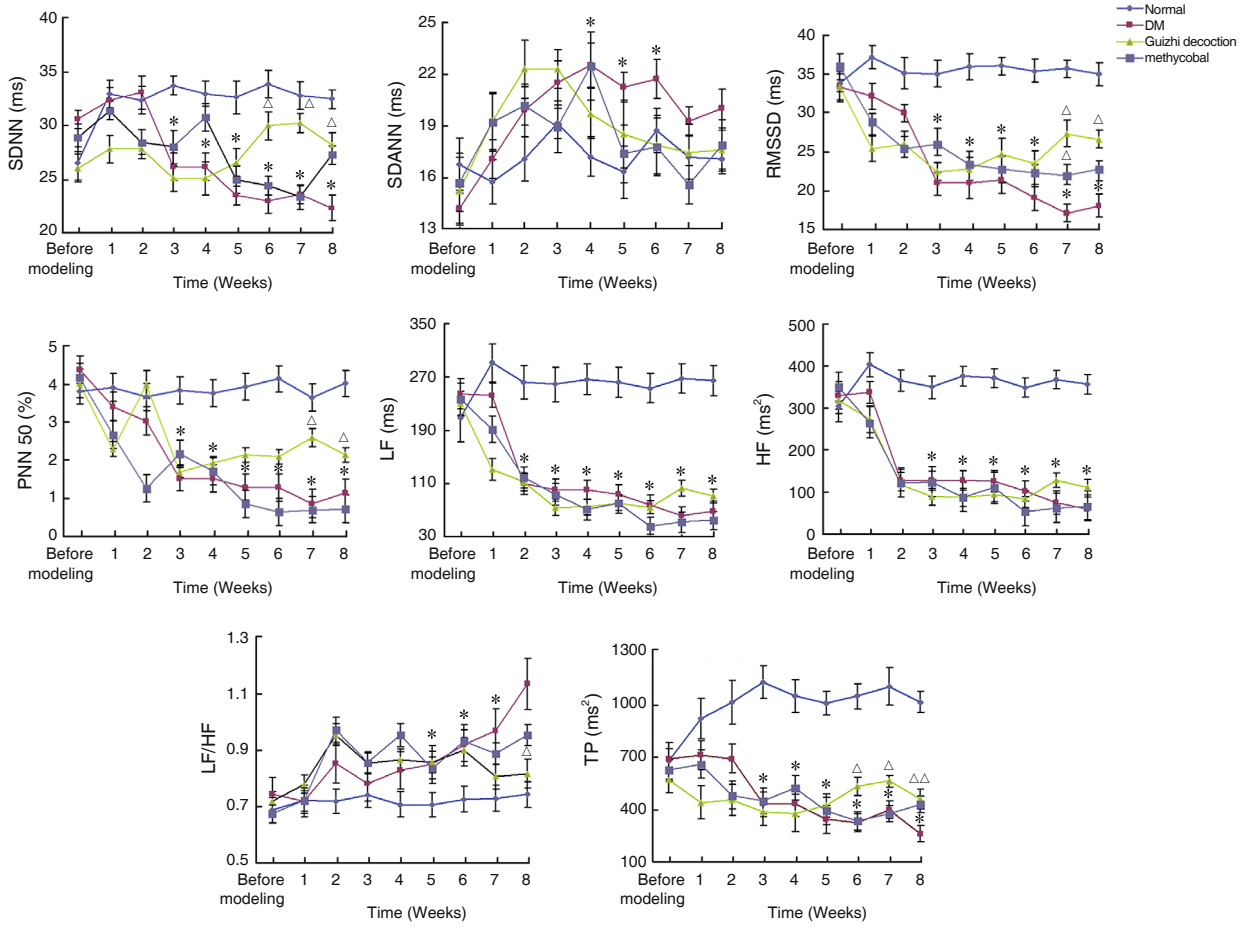


Figure 1. Changes of HRV in Diabetic Rats

Notes: *P<0.05, compared with the normal group; △P<0.05, compared with the model group

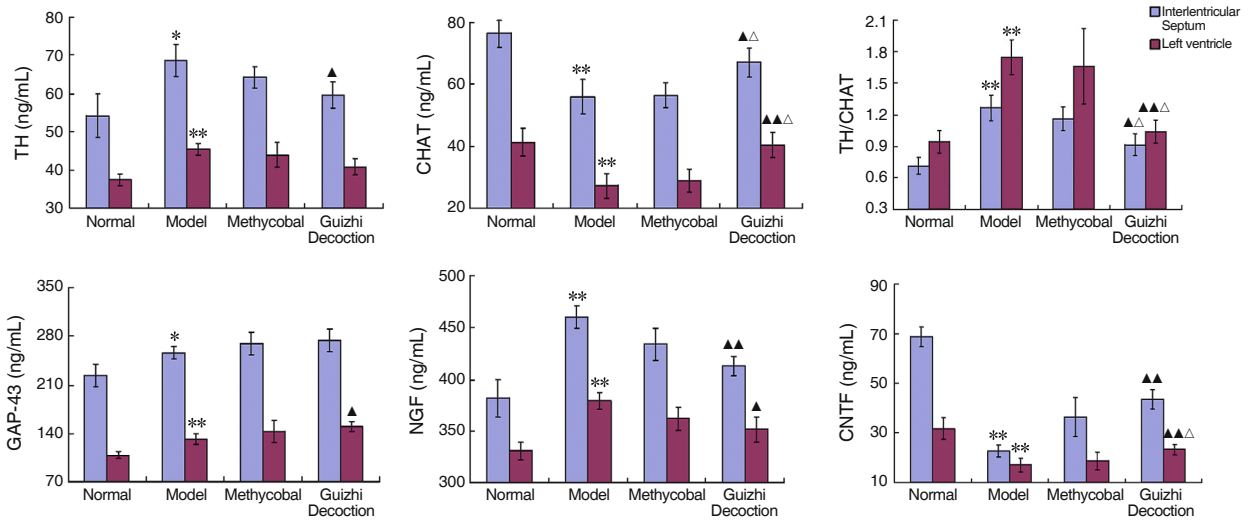


Figure 2. Changes in TH and CHAT Concentrations in the Interventricular Septum and Left Ventricle

Notes: $^{*}P<0.05$, $^{**}P<0.01$, compared with the normal group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$, compared with the model group; $^{\blacktriangle}P<0.05$, $^{\blacktriangle\blacktriangle}P<0.01$, compared with the methycobal group

TH in the left ventricle ($P<0.05$), and Guizhi Decoction reduced TH, elevated CHAT ($P<0.05$). Guizhi Decoction produced larger increases in CHAT and decreases in TH/CHAT than those in the methycobal group ($P<0.05$).

Changes in the Concentrations of GAP-43, NGF, and CNTF in the Left Ventricle and Interventricular Septum

The concentrations of neurotrophic factors in the myocardial homogenate of the interventricular septum and left ventricle were measured to observe changes in the local microenvironment during the regeneration process of diabetic CAN damage, thus suggesting factors that may regulate the imbalance of CANs. As shown in Figure 2, after STZ injection, the concentration of neurotrophic factors in the left ventricle and interventricular septum of rats significantly changed: GAP-43 and NGF increased, and CNTF decreased ($P<0.05$). Compared with the model group, only the CNTF concentration in the interventricular septum increased in the methycobal group ($P<0.05$). In the Guizhi Decoction group, GAP-43 increased, NGF decreased, and CNTF increased ($P<0.05$). Guizhi Decoction had significantly better effect than methycobal in regulating neurotrophic factors in the heart; there were significant differences in the reduction of NGF and increase of CNTF in Guizhi Decoction group ($P<0.05$).

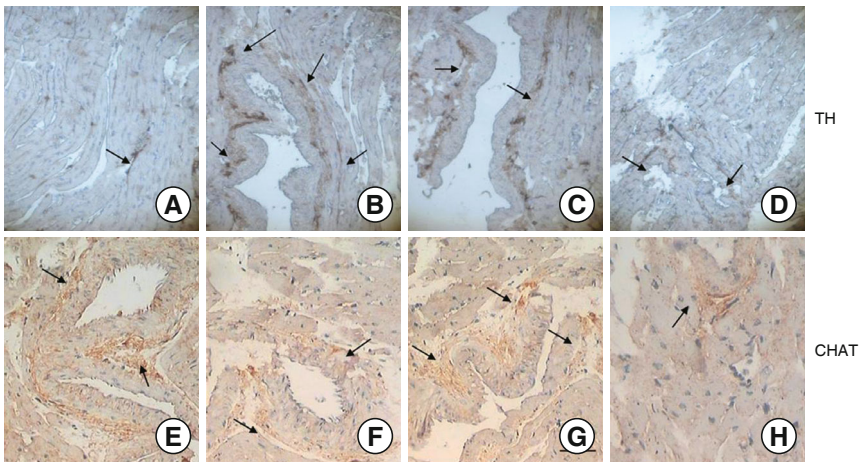
Changes in the Expression of TH and CHAT in the Right Atrium

As shown in Figure 3, 8 weeks after STZ injection to establish the model, the expression of TH in rat myocardia significantly increased, and the expression of CHAT significantly decreased ($P<0.05$). Compared with the model group, the methycobal group did not show significant differences, whereas TH expression decreased and CHAT expression increased significantly in rat myocardia in the Guizhi Decoction group ($P<0.05$).

DISCUSSION

Frontoni, et al[10] found that approximately 34% of type 2 diabetes patients had at least two abnormal readings in the cardiac autonomic function test. Maser, et al[11] summarized data collected between 1990 and 2001 and showed that the 5-year mortality rate of diabetic patients with CAN diseases was 53%, which was 3.3 times that of diabetic patients without CAN diseases. Therefore, the search for drugs that can treat diabetic CAN diseases is an important research direction.

HRV is a sensitive and quantitative indicator for the prediction of autonomic nerve function.[12,13] PNN50 is mainly determined by the tone of the vagus nerve, whereas the SDANN mainly reflects sympathetic nerve tone. In the frequency domain analysis, LF is a common indicator of the sympathetic and vagus nerves and indicates sympathetic nerve dominance; HF reflects vagus nerve tone,[14,15] and LF/HF reflects sympathetic-vagal balance. Currently, there are many studies regarding HRV in STZ rats. Schaan, et al[16] showed that the RMSSD of STZ rats was lower than that of the normal group and this HRV change was negatively correlated with blood glucose concentration.



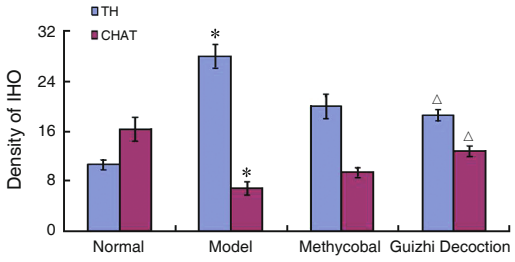


Figure 3. Immunohistochemistry of TH and CHAT in the Right Atrium of Diabetic Rats

Notes: Bar: 50 μm; A, E: normal group; B, F: model group; C, G: methycobal group; D, H: Guizhi Decoction group; the arrows meant the immunohistochemical positive tissues; $^{*}P<0.05$, compared with the normal group; $^{\triangle}P<0.05$, compared with the model group

Lin, et al[17] found that LF, TF, and LF/HF decreased in STZ rats at week 4, but HF was not statistically different from that of the normal control group. Lo, et al[18] showed that HF decreased 50% and LF decreased 70% in STZ rats. Howarth, et al[19] showed that SDANN decreased to its lowest level at week 4 of STZ injection and was not different from that of the normal control group at week 22. HF in STZ rats was lower than in the normal control at weeks 4–8. LF/HF was higher than in the normal control and continued until week 22, after which there was no difference from the normal control. These results showed that the decrease of HRV at the early stage of diabetes is mainly due to reduced cardiac vagal function and the destruction of sympathetic-vagal balance.

Our continuous 8 weeks of HRV studies in STZ rats showed that SDNN, RMSSD, and PNN50 in the model group continued to decrease after 3–4 weeks of model establishment, whereas SDANN began to increase but was not statistically different from that of the normal group. Analysis of the frequency domain showed that LF, HF, and TP rapidly decreased at weeks 1–2; afterwards, they continued to decrease and were significantly different from the normal group. LF/HF was slightly higher than that of the normal group. These results indicated that in the process of STZ-induced diabetes, the vagus nerve and sympathetic nerve were both injured, the damage of the vagus nerve was more serious, the sympathetic nerve was relatively hyperactive, there was sympathetic-vagal imbalance, and the disease presented as a decrease of SDNN and an increase of LF/HF.

TH is a marker of sympathetic nerve activity,[20] and CHAT is a marker of cholinergic neurons.[21] Bitar, et al[22] showed that TH increased by 52% in the myocardium after 60 days in STZ rats. Subsequently, Otake, et al[23] used TH immunohistochemistry to confirm that sympathetic nerve density increased in diabetic myocardium and that CHAT positive density did not change, thus causing electrophysiological disorder. Mabe, et al[24] showed that during the process of diabetes, atrial cholinergic neurons and nerve fibers were lost, whereas nerve density increased in the sinoatrial node, which was thought to be a compensatory response for atrial cholinergic neuron damage.

Therefore, due to the influences of long-term high blood glucose, the ultrastructure and function of CANs and nerve fibers were damaged, and their distribution density in the heart changed,[25-27] thus causing a decrease in vagus nerve function and a relative increase in sympathetic nerve function and resulting in sympathetic-vagal imbalance and loss of normal

regulation of the heart by the autonomic nerve.[28, 29]

The damage, repair, and regeneration of the autonomic nerve are associated with many neurotrophic factors. GAP-43 is mainly distributed in the presynaptic membrane, and its level is the highest in axon terminals during growth, development, and regeneration.[30-32] NGF is synthesized in tissues innervated by sympathetic and sensory nerves and plays a nutritional role in neurons through retrograde axonal transport.[33,34] CNTF plays a very important role in synapse formation between the vagus nerve and the myocardium and in the formation of postsynaptic function;[35] it also has a repair function after vagus nerve damage.[36] Therefore, we considered that the increase of NGF suggested sympathetic nerve damage and the presence of regeneration and remodeling.

This study showed that GAP-43 increased in the interventricular septum and left ventricle in STZ rats at week 8, indicating that CAN damage started being repaired. The increase in NGF indicated that the sympathetic nerve had a stronger repair capacity, whereas the significant decrease in CNTF indicated that the vagus nerve had more severe damage or insufficient repair capacity. Immunohistochemistry results showed that TH concentration increased, CHAT decreased, and TH/CHAT increased, indicating that sympathetic nerve density increased and vagus nerve density decreased. Therefore, we considered that during the damage process, the sympathetic nerve and vagus nerve were both damaged, the regeneration of the sympathetic nerve was dominant, the damage of the vagus nerve was more serious, and there was sympathetic-vagal imbalance.

Methycobal promotes the recovery of degenerated Schwann cells and the regeneration of nerve fibers.[37] Methycobal increased CNTF in the interventricular septum and had mild regulation effects on GAP-43, NGF, TH, CHAT, and TH/CHAT. Guizhi Decoction increased GAP-43, significantly increased CNTF, and decreased NGF concentration, indicating that Guizhi Decoction better repaired the vagus nerve and inhibited sympathetic nerve regeneration. In the presentation of HRV, Guizhi Decoction increased SDNN, RMSSD, and PNN50; increased LF, HF, and TP; and decreased LF/HF in STZ rats.

Analysis of the results from this study showed that STZ diabetic rats had increased blood glucose, decreased body weight, and reduced heart rate. Guizhi Decoction did not significantly affect blood glucose or heart rate. These results also indicated that the protection and regulation of Guizhi Decoction on the autonomic nerve do not depend on the regulation of blood glucose.

A review of current literature showed that Guizhi Decoction had two-direction regulatory effects on sweat glands, body temperature, blood pressure, bowel movement, and immune function in experimental animals.[38] Treatment of myocarditis, coronary heart disease, arrhythmia, circulatory system diseases, and diseases that involve autonomic nerve disorders, and diabetic autonomic neuropathy with Guizhi Decoction or its modified formula was more effective.[39,40]

For sympathetic-vagal imbalance, we considered that pathogenesis could be summarized as "damage of collaterals by toxic heat will involve Ying and Wei; if Ying and Wei are not harmonious, then Heart (Xin) and mind will be consumed." Damage of Ying and Wei will definitely result in yin and yang imbalance in the Heart. The deficiency of Ying qi and blood and the damage of Ying and Wei will cause failure of nourishment of the Heart. Guizhi Decoction is the representative prescription for harmonizing Ying and Wei and balancing yin and yang. The vagus nerve is mainly distributed in the sinoatrial node, followed by the atrioventricular node, and the ventricle has the least distribution, whereas the sympathetic nerve is evenly distributed in the heart. Therefore, changes in the distribution of the sympathetic and vagus nerves in the right atrium have an important meaning. The results of present study indicated that Guizhi Decoction inhibited the regeneration of the sympathetic nerve in the right atrium, which helped maintain sympathetic-vagal balance.

Due to the difficulty of collecting material from the right atrium, the tissue from the right atrium was only used for immunohistochemistry; the detection of TH, CHAT, and neurotrophic factors was only performed on material from the interventricular septum and some of the left ventricle. We only observed the effects of treatment with Guizhi Decoction for 4 weeks and did not evaluate its long-term efficacy.

Guizhi Decoction was a better treatment and had superior repair function in the vagus nerve; Guizhi

inhibited the regeneration and the function of the relatively hyperactive sympathetic nerve and regulated autonomic nerve imbalance. The regulatory function of Guizhi Decoction in the autonomic nerve was independent of blood glucose regulation.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Li X designed the experiments, and drafted the manuscript. Jiang YH performed the experiments, analyzed the data analysis and drafted the manuscript. Jiang P collected and analyzed the data. Yang JL and Ma DF performed the animal care and telemetry. Yang CH revised the manuscript. All authors read and approved the final manuscript.

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(Received October 17, 2013)
Edited by GUO Yan