ORIGINAL ARTICLE

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

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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 electrocardiographic signals and all indicators 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 diabetes mellitus, cardiac autonomic neuropathy, sympathetic-vagal imbalance, heart rate variability, Chinese medicine, Guizhi Decoction

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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 \(\kappa B\) (NF-\(\kappa B\)), interleukin-1 (IL-1), tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), 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