

Effect of baihu ginseng decoction on treatment of type 2 diabetes.

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Abstract

Objective: To investigate the effects of baihu ginseng decoction on intestinal microflora structure in type 2 diabetic rats.

Methods: Total 60 type 2 diabetic SD rats were randomly divided into three groups, including model group, gliclazide group and baihu ginseng decoction group. In the gliclazide group, rats were treated with gliclazide lavage, while rats in baihu ginseng decoction group were treated baihu ginseng decoction lavage. After 2 w, the level of blood glucose, blood lipid, glucose tolerance, glycosylated Hemoglobin (HbA1c) and insulin in all rats were measured and analysed. Meanwhile, the change of intestinal microflora structure in stool samples were detected with quantitative PCR.

Results: Baihu ginseng decoction could help decrease the concentration of blood glucose, cholesterol and triglyceride obviously in diabetic rats, increase the diabetic rats' High Density Lipoprotein Cholesterol (HDL-C) and the levels of *Lactobacillus* and *Bifidobacterium*.

Conclusion: Baihu ginseng decoction has significant effects on reducing blood glucose and blood lipids. Its hypoglycemic effect may be related to the community of *Lactobacilli* and *Bifidobacterium*.

Keywords: Baihu ginseng decoction, Type 2 diabetes mellitus, Blood glucose, Blood lipids, Intestinal flora structure.

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Introduction

Diabetes Mellitus (DM) is a common chronic systemic endocrine and metabolic disease, which affects people's health seriously. With the development of social economy and life expectancy, the case rate of diabetes is rising. Diabetes mellitus and its complications have brought great harm and burden to society, and now it has become a worldwide public health problem [1,2]. Per different physiology, the diabetes mellitus can be classified into types 1 and 2 diabetes. Type 2 diabetes is primarily related to obesity and lack of exercise. The physiological symptoms of type 2 diabetes include high blood sugar, insulin resistance, and relative lack of insulin. However, the mechanism of type 2 diabetes is still unclear. Some researches demonstrated that change of DNA methylation caused by lifestyle and diet might be responsible. Recently, the microbiome structure was also connected to type 2 diabetes. Baihu ginseng decoction is always used to regulate the endocrine system in human body. Since the dysregulation of endocrine system in type 2 diabetes, we postulate that whether baihu ginseng decoction can be used to treat type 2 diabetes, which has never been investigated before. Meanwhile, we also investigate that effect of baihu ginseng decoction on microbiome structure which might explain the mechanism of its effect on treatment of type 2 diabetes.

Materials and Methods

Animals

Male SD rats, weighing 210 g-220 g, were purchased from the experimental animal center of West China Medical Center of Sichuan University. Adaptive feeding 5 d, eight rats were selected as the blank control group and were fed on ordinary feed, the others were fed with high glucose and high fat diet. After 4 w, all rats were intravenous injected of STZ under non-narcotic condition. After 2 w, model replication successfully rats were selected and randomly divided into model group, Gliclazide Tablets group (32 mg/kg/d) and Baihu Ginseng Decoction group (37.2 g/kg/d) with 8 rats in each group. The corresponding drugs were given respectively, and the blank group and the model group were treated with equal volume of normal saline for 2 w.

Main instruments and reagents

Process of making baihu ginseng decoction: According to the original proportion, gypsum 50 g, *Anemarrhena* 18 g, licorice 6 g, rice 9 g with 10 times water are decocted for 1 hour, then filtered, filter residue removed and decocted with 8 times distilled water for 2 times, filtered again, finally decocted together. 10 g red ginseng stewed, combined with the above decoction, filtered together, concentrated together 2.5 g/ml, placed at refrigerator with constant temperature of 4°C. Glycosylated Hemoglobin Cholesterol (HbA1c-C), Triglyceride

(TG), Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), blood Glucose (Glu) kit (batch number respectively 2812506, 3347854, 108201E, 112568D, 994683E. Beijing Lideman biochemical Limited by Share Ltd., China), insulin (Ins) kit (lot number 1125687, Shenzhen Sai'er Biotechnology Co., China). Fluorescence quantitative PCR instrument ABI7500 System (ABI company, USA), SniartSpecTM3000 ultraviolet spectrophotometer (Bio-Rad, USA), gel imager (Bio-Rad, USA), SW-CJ-2FD type double-sided cleanbench (Suzhou purification equipment Co., China), Mastercycler gradient PCR (Eppendorf company, USA), the level of electrophoretic instrument (Shanghai Jing Yi Glass Instrument Factory, China), Himac CR22G high speed refrigerated centrifuge (FI the Hitachi company, Japan), centrifuge (Eppendorf, USA), rTaq DNA polymerase (TaKaRa, China), DL2000 DNA Marker (Takara, China), SYBR Premix DimerErase (Takara, China), bacterial genomic DNA extraction kit (TIANGEN, China), agarose gel recovery Kit (TIANGEN, China).

Index monitoring

Fasting blood glucose measurement: Samples of rat blood were collected before model replication, after model replication and 2 w after administration. Rats in each group were fasted for 12 h and then their tails were cut in the absence of anesthesia to get their blood to measure Hemoglobin Cholesterol (HbA1c-C), Triglyceride (TG), Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), blood Glucose (Glu) and Insulin (Ins) followed by the observation on the changes of blood glucose if rats in each group.

Samples of rat feces were collected before model replication, after model replication and 2 w after administration. Fecal samples of rats were collected above 2 g and stored at the temperature of -70°.

Pretreatment of fecal samples: Ever sterile centrifuge tube was put in 2 g wet fecal samples and 20 ml PBS (0.01 mol/L, pH 7.4) and then fully oscillating mixed until the sample turned into uniform turbidity without large condensation except the insoluble residue of food. 3000 rpm centrifuge for 5 min. The supernatant was collected and precipitation was discarded and washed for three times. The supernatant was centrifuged 10 min at 12000 rpm, the supernatant was discarded and sediment was collected. The bacteria collected were sterilized with 20 ml PBS (pH 7.4) suspension, 12000 rpm centrifugal 5 min and washed for 3 times. The washed cells mixed with 2 ml sterile water and were divided into two tubes of 1.5 ml, 12000 rpm centrifugal 5 min.

Extraction of total bacterial DNA from fecal bacteria: Bacterial genomic DNA extraction Kit was used to extract bacterial total DNA. And specific steps are performed according to operation instructions.

Quantitative PCR

According to the sequence of *16SrRNA* gene, primer design software Primer Premier 5 was used to design specific PCR primers. And their corresponding species specificity of primer sequences was compared in BLAST gene library. The genomic DNA of the blank group was used as the template, and the conventional PCR reaction was performed with different primers. The routine PCR products of the target bacteria were recovered by DNA purification kit, and the operation was carried out according to the kit instruction. The conventional target bacteria PCR products were recovered after the DNA fragment as a standard process of fluorescence quantitative PCR. UV spectrophotometer was used to determine the A260 value. According to the relationship between the A260 value and the fragment length, it was converted for each standard copy number of/ml and used for the production of standard curve. Standard curve making: each standard would be diluted into 10 times series, and each 10 times dilution concentration for eight ones. The standard curve of *Lactobacillus planetarium* and its specific strains was automatically generated by using ABI7500 System data analysis software according to the signal of the signal amplification during the reaction of the diluted standard products.

Statistical analysis

The data were processed by IBM, SPSS 17.0 statistical software. The measurement data were checked by t-test and the count data chi-square test. Test level $\alpha=0.05$, $P<0.05$ suggested the difference is statistically significant.

Results

Effects of baihu ginseng decoction on Glu, Ins and HbA1C

Baihu ginseng decoction can reduce rats' Glu and HbA1c ($P<0.05$) obviously. Compared with the model group, the Glu and HbA1c of the baihu ginseng decoction group were lower than that of the model group, which showed the baihu ginseng decoction had a better hypoglycemic effect ($P<0.05$). Baihu ginseng decoction had no significant influence on serum Ins content in diabetic rats ($P>0.05$, Table 1).

Effects of baihu ginseng decoction on TG, TC and HDL-C

Baihu Ginseng decoction can obviously reduce the content of TG and TC in diabetic rats ($P<0.05$), and increase the content of HDL-C ($P<0.05$, Table 2).

Results of two kinds of fecal flora by Q-PCR quantitative detection

After the success of modeling, the blank group, model group, gliclazide tablets group and baihu ginseng soup group of fecal *Lactobacilli* and *Bifidobacteria* decreased significantly

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(P<0.05). After 2 w of administration, gliclazide tablets group of fecal *Lactobacilli* and *Bifidobacteria* had no obvious change, while those of baihu ginseng soup were significantly

increased. Compared to before treatment and model group, the difference was statistically significant (P<0.05).

Table 1. Effects of baihu ginseng decoction on Glu, Ins and HbA1C.

	Before model replication				After model replication				2 w after administration			
	Blank group	Model group	Gliclazide group	Baihu ginseng group	Blank group	Model group	Gliclazide group	Baihu ginseng group	Blank group	Model group	Gliclazide group	Baihu ginseng group
Glu (mmol/L)	4.20 ± 1.35	4.15 ± 1.50	4.11 ± 1.40	4.19 ± 1.30	4.25 ± 2.25	19.45 ± 5.20 [†]	18.32 ± 2.50 [†]	19.05 ± 5.55 [†]	4.50 ± 2.50	19.45 ± 5.20	6.32 ± 2.11 [#]	7.18 ± 1.92 [#]
Ins (mU/L)	27.84 ± 6.38	28.13 ± 5.50	27.90 ± 5.35	28.54 ± 6.56	27.75 ± 5.35	63.26 ± 9.15 [†]	69.42 ± 13.17 [†]	54.29 ± 8.84 [†]	27.77 ± 7.10	54.19 ± 5.42	54.27 ± 11.36	51.27 ± 11.22
HbA1C (%)	18.66 ± 5.32	17.56 ± 4.55	15.78 ± 3.15	16.90 ± 3.85	17.56 ± 4.45	64.12 ± 15.5 [†]	63.90 ± 14.59 [†]	64.22 ± 16.80 [†]	19.10 ± 5.50	72.15 ± 15.3	48.22 ± 10.17 [#]	52.33 ± 12.76 [#]

Note: Compared with the blank group, [†]P<0.05; Compared with the model group, [#]P<0.05.

Table 2. Effects of baihu ginseng decoction on TG, TC and HDL-C.

	Before model replication				After model replication				2 w after administration			
	Blank group	Model group	Gliclazide group	Baihu ginseng group	Blank group	Model group	Gliclazide group	Baihu ginseng group	Blank group	Model group	Gliclazide group	Baihu ginseng group
TG (mmol/L)	0.43 ± 0.12	0.45 ± 0.15	0.41 ± 0.10	0.40 ± 0.20	0.35 ± 0.15	0.89 ± 0.31 [†]	0.91 ± 0.30 [†]	0.90 ± 0.29 [†]	0.45 ± 0.15	0.91 ± 0.20	0.56 ± 0.11 [#]	0.56 ± 0.23 [#]
TC (mmol/L)	1.19 ± 0.12	1.18 ± 0.15	1.17 ± 0.13	1.18 ± 0.12	1.17 ± 0.35	2.68 ± 0.20 [†]	2.76 ± 0.25 [†]	2.57 ± 0.10 [†]	1.18 ± 0.1	2.69 ± 0.13	1.05 ± 0.13 [#]	1.36 ± 0.25 [#]
HDL-C (mmol/L)	1.87 ± 0.30	1.85 ± 0.25	1.91 ± 0.29	1.88 ± 0.27	1.87 ± 0.30	1.12 ± 0.15 [†]	1.13 ± 0.16 [†]	1.14 ± 0.15 [†]	1.90 ± 0.5	1.12 ± 0.24	1.54 ± 0.17 [#]	1.41 ± 0.16 [#]

Note: Compared with the blank group, [†]P<0.05; compared with the model group, [#]P<0.05.

Table 3. Effects of baihu ginseng decoction on fecal *Lactobacilli* and *Bifidobacterium*.

	Before model replication				After model replication				2 w after administration			
	Blank group	Model group	Gliclazide group	Baihu ginseng group	Blank group	Model group	Gliclazide group	Baihu ginseng group	Blank group	Model group	Gliclazide group	Baihu ginseng group
<i>Lactobacillus</i>	5.07 ± 1.55	5.04 ± 1.78	5.06 ± 1.77	5.07 ± 1.76	5.08 ± 1.78	3.97 ± 1.21	4.08 ± 1.43	3.95 ± 1.56	5.07 ± 1.55	4.09 ± 1.54	3.97 ± 1.56	4.95 ± 1.44
<i>Bifidobacterium</i>	4.55 ± 2.73	4.59 ± 2.77	4.56 ± 2.74	4.55 ± 2.75	4.57 ± 1.55	3.56 ± 1.73	3.55 ± 1.73	3.55 ± 1.75	4.57 ± 2.55	3.07 ± 1.55	3.07 ± 1.65	4.47 ± 0.69

Discussion

The basic pathophysiology of type 2 diabetes mellitus is the metabolic disorder caused by absolute or relative deficiency of insulin and glucagon activity increased. Its pathogenesis mainly exists in insulin resistance and insulin secretion disorder based on gene defects. However, new researches have suggested that intestinal flora imbalance may be an important cause of diabetes. According to the detection and analysis of patients with type 2 diabetes and healthy people feces, early researchers found that the water content in the stool of patients decreased significantly and the content of intestinal decaying substance and toxin were obviously higher than that of normal

people, which overweighed the metabolic burden for patients. Moreover, the number of beneficial bacteria in patient's body were reduced, especially the number of *Bifidobacterium*. While the quantity of harmful bacteria was relatively increased. Once the number of harmful bacteria exceeded a certain range, it may cause infection (including infection of the skin, mucous membranes, organs and blood), and infection was one of the reasons that caused the complications of diabetes [2]. The intestinal flora can degrade some of the food components (such as Grain β-, dextran, etc.) that human cannot digest by themselves (such as cereals β-, beta glucan, etc.), which produced oligosaccharides or monosaccharides, and short chain fatty acids, etc. According to the analysis of esophageal

flora in type 2 diabetic mice and normal mice, it was found that the normal mice contained *Lactobacillus* in the esophagus, while the mice in the diseased esophagus contained no or quite low *Lactobacillus*. In recent years, Chinese researchers conducted a two-phase macro genome correlation analysis of 345 Chinese enteric microorganisms. They had identified about 60,000 molecular markers associated with type 2 diabetes and made the intestinal micro biological composition difference between China diabetic and nondiabetic patients clear in the molecular level. And they had found that there existed antagonistic relationship between bacteria and harmful bacteria, which especial stood out obviously among the different bacterial flora of *Clostridium* [3].

Although there was no direct relationship between intestinal bacteria and diabetes, probiotics can play a role in alleviating diabetes when the beneficial microflora were predominant. Probiotics can improve diabetes by reducing glucose absorption in the gastrointestinal tract. If probiotics adhered to the gastrointestinal tract, they would consume the glucose, thus reducing glucose that delivered to the body and reducing glucose levels in the blood. In addition, when there were many intestinal bacteria existed, these bacteria consumed a larger amount of food, which reduced the amount of food the body absorbed relatively and decreased greatly the body glucose intake. Therefore, it helped to reach the goal of controlling the blood glucose [4]. In addition, gut microbiota can also affect hormone levels and indirectly affect the development of diabetes. Researchers can assess intestinal microbiota to understand the association between hormonal changes and diabetes in different populations [5]. After the treatment with adequate *Lactobacillus* GMNL-263 (Lr263) for fourteen weeks, the insulin resistance of type II diabetic rats was decreased, and their hepatic steatosis was significantly improved [6]. Therefore, intestinal flora has important value and broad application prospect in the prevention and treatment of diabetes mellitus.

Chinese medicine is a great treasure house. Traditional Chinese medicine is simple, convenient, cheap and effective. If we can dig out and improve the recipe for effective treatment of diabetes from the great treasure of Chinese medicine. This will have a great significance in reducing the cost of treatment, the medical costs, medication side effects for patients and patient's suffering.

At present, many kinds of Chinese medicine have been proved to play a positive role in the treatment of diabetes. Baihu ginseng decoction comes from Shanghan Zabing Lun, which was an effective clinical prescription. Modern scholars have studied the clinical efficacy of baihu ginseng decoction, and have confirmed that the formula has a definite effect on reducing blood glucose, glycosylated hemoglobin, blood lipids, and improving insulin resistance in patients with type 2 diabetes mellitus [7-11]. Zhao et al. [12,13] believed that its hypoglycemic effect may be related to its anti-oxygen free radicals, the protection of pancreatic β cells and the increase of insulin sensitivity in rats. Lai et al. [14] found the baihu added with ginseng decoction could significantly increase insulin

sensitive index and it had obvious protective effect on islet function in type 2 diabetic rats with insulin resistance. Then it was further suggested that the mechanism may be closely related with regulating the expression of GLUT4, Ins and maintaining the normal structure and function of pancreatic islet cells. In clinical practices, more and more scholars apply baihu ginseng decoction to treat patients with type 2 diabetes, which can improve clinical symptoms of patients significantly by reducing fasting blood glucose in patients after meal, blood glucose and glycosylated hemoglobin with a great clinical curative effect [15,16].

The results of this study showed that the model group, the Glu, HbA1c, Ins, TG, TC and HDL-C in Model group, gliclazide tablets group and baihu ginseng soup group were significantly increased after replication ($P < 0.05$), suggesting that the model of this research was successful. Besides, compared with the blank control group, the model group, gliclazide tablets group and baihu ginseng decoction group of fecal *Lactobacilli* and *Bifidobacterium* decreased significantly ($P < 0.05$), suggesting that the diabetes restrained the intestinal bacteria in the intestinal tract of rats. 2 w after administration, the Glu and HbA1c of the baihu ginseng decoction group were lower significantly than that of the model group ($P < 0.05$), which showed it had a good hypoglycemic effect. In addition, the content of TG and TC in rats with baihu ginseng decoction decreased obviously, and their content of HDL-C increased significantly ($P < 0.05$), suggesting that it had a better effect on reducing blood lipids. Compared with the previous administration and the model group, the fecal *Lactobacilli* and *Bifidobacterium* of the baihu ginseng decoction group were significantly increased, and the difference was statistically significant ($P < 0.05$), suggesting that the baihu ginseng decoction can promote intestinal *Bifidobacterium* and *Lactobacillus* in reproduction.

To sum up, baihu ginseng decoction plays a significant role in reducing blood sugar and reducing blood lipids and its hypoglycemic effect may be related to the community of *Lactobacillus* and *Bifidobacterium*.

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