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Effects of Metformin on Gut Microbiota and Dyslipidemia in Type 2 Diabetic Rats

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Abstract

To explore the effects of metformin on the composition of gut microbiota in type 2 diabetes mellitus (T2DM) rats and its intervention on dyslipidemia. Male Sprague-Dawley rats were randomly divided into normal control group, T2DM group and metformin-treated T2DM group, with 8 rats in each group. The T2DM model was established by streptozotocin plus high-fat and high-sugar diet. The metformin-treated T2DM group was treated with metformin by gavage for 8 weeks. Oral glucose tolerance test (OGTT) was performed in the three groups. Fasting blood glucose level was measured by glucomytometer, fasting serum insulin level was measured by ELISA, and serum alanine aminotransferase, aspartate aminotransferase, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglyceride levels were detected. Fecal DNA was extracted and gut microbiota was analyzed by 16S rRNA gene sequencing. The results showed that metformin significantly increased the body weight of T2DM rats, decreased liver index, liver enzyme activities, insulin resistance index, and alleviated dyslipidemia in T2DM rats. In addition, metformin reduced the ratio of Firmicutes/Bacteroidetes in T2DM rats. Metformin significantly increased the abundance of Phascolarctobacterium, Streptococ-cus, Lachnoclostridium, and Parasutterella in T2DM rats.

Keywords

Metformin; Type 2 diabetes mellitus; Gut microbiota; Blood lipids.

1. INTRODUCTION

Diabetes is a chronic metabolic disease with a rapidly increasing prevalence, most of which are type 2 diabetes mellitus (T2DM)[1]. Studies have proved that there is a link between T2DM and gut microbiota. The imbalance of intestinal flora may promote metabolic endotoxemia and systemic inflammation, leading to the development of insulin resistance, thereby increasing the risk of T2DM[2]. In vitro and clinical studies have shown that metformin has promising antidiabetic effects. Recent studies have shown that metformin interacts with gut microbiota and its metabolic derivatives, such as short-chain fatty acids, and plays an important role in improving metabolic-related diseases[3]. Although intestinal microbiota has become a new target for the prevention and treatment of T2DM and related metabolic diseases, the current research on the changes of intestinal microbiota in T2DM patients with metformin is limited, and further exploration and research are still needed[4]. Therefore, the aim of this study is to investigate the effect of metformin on streptozocin plus high-fat-high-sugar diet-induced T2DM rats, to explore the possible role of intestinal microbiota in metformin treatment, and to better understand the molecular mechanism of metformin treatment.

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2. MATERIALS AND METHODS

2.1. Main reagent

The metformin, streptozocin, and rat insulin ELISA kits were purchased from Sigma, and the Qiagen Fecal DNA Rapid Extraction kit was purchased from Qiagen. Colorimetric detection kits for alanine aminotransferase, aspartate aminotransferase, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were purchased from Nanjing Jiancheng BioEngineering Institute.

2.2. Animals were grouped with the experiment

Thirty male Sprague-Dawley rats (8 weeks old, 180-200 g) were purchased from Vitong Liwa Laboratory Animal Technology Co., LTD. They were housed in specific pathogen free (SPF) animal room with 12 h light/dark cycle, stable room temperature and relative humidity, and fed and drinking freely. After 1 week of adaptive feeding, the rats were divided into two groups by random number table method. normal control (NC, n = 8) group, fed with laboratory standard diet. T2DM group (n = 22) : animals were fed with a high-fat and high-sugar diet for 4 weeks, fasted for 12 hours, and then injected with 30 mg/kg GSTZ (in 0.1 mol/L citrate buffer, pH 4.5) via the tail vein. After 72 hours, blood was collected from the tail vein by acupuncture, and blood glucose was detected by glucometometer. The blood glucose level exceeding 11.1 mmol/L was defined as successful modeling. Rats in NC group were injected with the same volume of citrate buffer via the tail vein. Sixteen rats in T2DM group were randomly selected and divided into 2 subgroups: T2DM+ metformin group (n =8) and T2DM group (T2DM+ normal saline, n =8), which were given metformin (100 mg/kg) and the same amount of normal saline by gavage for 8 weeks, respectively.

2.3. Oral glucose tolerance test was performed

At the end of the experiment, oral glucose tolerance test was performed after fasting for 12 h, and glucose (2 g/kg body weight) was given by gavage. Blood was taken from the tail of the rats before and at 30, 60, 90, and 120 min after the glucose load, and blood glucose levels were recorded with a glucomytometer.

2.4. Biochemical measurements

After 8 weeks of intervention, the rats were fasted for 12 hours, and the orbital blood was collected after anesthesia. Serum was quickly separated and stored in the refrigerator at －80 ℃ for biochemical indexes detection. fasting bloodglucose (FBG) was measured with a glucometer, and fasting serum insulin was measured with rat insulin ELISA kit. Serum ALT, AST, TC, HDL-C, LDL-C, and TG were measured using commercial kits.

2.5. Gut microbiota analysis

DNA was extracted from total fecal bacteria using the Qiagen Fecal DNA Rapid Extraction Kit according to the manufacturer's instructions. Universal primers (341F: 5'-ACTCCTACGGGAGGCAGCAG-3', 806R: 5'-GGACTACHVGGG-TWTCTAAT-3') were used to amplify the hypervariable region V3-V4 of microbial 16S rRNA. The amplicon library was quantified using the KAPA HiFi Hotstart PCR kit using a Qubit 2.0 fluorometer and then sequenced on an Illumina HiSeq 2500 platform for 250-bp pairedend reads. USEARCH (v7.0.1090) software was used to generate operation-al taxonomic units (OTUs) by clustering labels with 97% similarity. The final OTUs were classified based on the RDP classifier algorithm using the GreenGene database. QIIME was used to analyze alpha diversity and beta diversity.

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2.6. Statistical analysis

All statistical analysis were performed using SPSS 20.0 software. One-way analysis of variance and Tukey's post hoc test were used to analyze the differences between groups when the data were normally distributed and had homogeneity of variance. Otherwise, the Kruskal-Wallis test and the Mann-Whitney test were applied. The test level $\alpha = 0.05$.

3. RESULTS

3.1. Effects of metformin on body weight, liver index and liver enzyme activity in T2DM rats

The results in Table 1 show that compared with the NC group, the rats in the T2DM group had a decrease in body mass and a significant increase in liver index and liver enzyme activities (ALT and AST) (all $P < 0.05$). Compared with the T2DM group, the body weight of the T2DM+ metformin group was significantly increased ($P < 0.05$), and the liver index and liver enzyme activities (ALT and AST) were significantly decreased (all $P < 0.05$).

Table 1. The impact of metformin on body mass, hepatic index, serum ALT and AST activities in T2DM rats

Group	body mass (g)	liver index (%)	ALT (U/L)	AST (U/L)
Control	511.25±8.35	2.53±0.20	129.35±11.34	65.22±10.21
T2DM	455.65±7.22	4.88±0.75	452.65±26.24	102.21±25.63
Metformin+T2DM	535.86±12.98	3.56±0.66	301.25±18.12	84.21±18.55

3.2. Effects of metformin on FBG and blood lipids in T2DM rats

As shown in Table 2, Compared with the NC group, FBG levels were increased in T2DM rats , and metformin reduced FBG levels in T2DM rats ($P < 0.05$). The T2DM group rats developed dyslipidemia, which was manifested as significantly increased serum TC, TG, and LDL-C levels (all $P < 0.05$) and significantly decreased serum HDL-C levels ($P < 0.05$), while metformin reduced dyslipidemia in T2DM rats.

Table 2. The effects of metformin on metabolic parameters in T2DM rat

Group	FBG (mmol/L)	Insulin (ng/mL)	TC (mmol/L)	TG (mmol/L）	HDL-C (mg/dL)	LDL-C (mg/dL)
Control	4.98±0.35	0.89±0.20	1.22±0.24	0.55±0.11	0.88±0.25	0.38±0.08
T2DM	18.54±1.57	1.78±0.75	2.84±0.54	1.56±0.57	2.55±0.71	1.25±0.15
Metformin+T2DM	9.54±0.98	1.16±0.54	1.81±0.41	1.02±0.25	2.57±0.56	0.98±0.12

3.3. Effects of metformin on the characteristics of intestinal microbiota in T2DM rats

The Alpha diversity results (Table 3) showed that the intestinal microbiota richness of the T2DM group was lower than that of the NC group, and the intestinal microbiota richness of the T2DM+ metformin group was higher than that of the T2DM group, but the differences were not statistically significant (both $P > 0.05$). As shown in FIG. 2, PCoA was performed on the unweighted UniFrac distance matrix in order to assess the effect of metformin on the gut microbiota of T2DM rats. The first two principal coordinates of PCoA (component 1 and component 2) were divided into NC group, T2DM group, and T2DM+ metformin group, and the T2DM group and T2DM+ metformin group shared overlapping regions . T2DM group and T2DM+ metformin group showed similar differences in alpha and beta diversity of intestinal microbiota (all $P > 0.05$).

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Table 3. Alpha diversity indices

Group	OTUs	Chao1	Shannon	Simpson	PD_whole_tree
Control	1536.21±465.15	4520.15±365.51	120.88±14.24	7.55±1.21	0.88±0.45
T2DM	1035.32±336.57	3758.50±363.25	72.25±8.54	5.54±0.57	0.89±0.71
Metformin+T2DM	1199.54±405.98	3987.55±401.25	98.51±12.55	6.22±0.45	0.88±0.54

4. CONCLUSION

In this study, a high-fat diet and STZ injection were used to simulate the characteristics of human T2DM, including hyperglycemia, hyperinsulinemia, and dyslipidemia; this animal model of T2DM has been used in several studies. We found that metformin effectively alleviated glucose tolerance, insulin resistance, and dyslipidemia in the T2DM rat model. Studies have demonstrated the antihyperglycemic effects of metformin in various animal models, such as obese rodents, STZ-induced diabetic rats, or STZ-nicotinamide induced diabetic rats.

The gut microbiome is emerging as a promising target for the treatment or prevention of inflammatory and metabolic disorders in humans[5]. Alteration of intestinal microbiota composition by metformin is considered to be one of the main mechanisms of its action in vivo, and it has been suggested that metformin-mediated microbiota is the driving force for improving renal function, improving glucose homeostasis, restoring intestinal permeability, and reducing inflammatory markers[6-8]. Several studies have shown that the gut microbiota of obese animals and humans exhibit a higher ratio of Firmicutes/Bacteroidetes compared to individuals of normal body mass[9-11]. The ratio of Firmicutes to Bacteroidetes is considered an indicator of gut microbial imbalance associated with a high-fat diet, Bacteroidetes was positively associated with fat but negatively associated with fiber, while Firmicutes showed the opposite association. Our results showed that the ratio of Firmicutes/Bacteroidetes was increased in the fecal microbiota of T2DM rats, which was reduced by metformin. Previous studies have reported that the ratio of Firmicutes/Bacteroidetes is positively associated with the incidence of obesity and diabetes. Similarly, fecal microbiota analysis in diabetic nephropathy mice showed that metformin caused gut microbiota remodeling, increasing the abundance of Bacteroidetes and decreasing the abundance of Firmicutes, and this trend in Firmicutes levels was similar to the metformin-mediated microbiota changes in T2DM rats.

Taken together, our study supports the hypothesis that metformin induces alterations in the gut microbiota composition of diabetic rats and is responsible for the antidiabetic effects of metformin. In particular, metformin improved the abundance of beneficial gut microbiota, including those producing SCFAs. This study is helpful to enrich the research on the mechanism of metformin intervention of intestinal microbiota in T2DM and related metabolic diseases, strengthen the theoretical basis of metformin intervention of metabolic syndrome, and provide ideas for the clinical identification of probiotics for the treatment of metabolic diseases.

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