Effects of Polysaccharide MDG-1 from Ophiopogon Japonicus on Insulin Resisitance and Inflammatory Factors in T2DM Rats

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Abstract: To investigate the effects of Polysaccharide MDG-1 from Ophiopogon japonicus on glucose and blood lipid, insulin resistance and the levels of inflammatory factors in T2DM rats. The T2DM rat model induced by intravenous injection small dosage of streptozotocin(STZ) and feeding high fat-high glucose diet. The rats whose glucose level≥11.1mmol·L^{-1} were randomly divided into 5 groups(n=10 in each): model group, glibenclamide group, Polysaccharide MDG-1 from Ophiopogon japonicus of high dose, medium dose and low dose. Five weeks later, to detected the FBG, blood lipid, TG, CHOL, HDL-C and LDL-C, INS, TNF-α and CRP in the serum were detected by the method of radioactive immunoassay, and accordingly INS and FBG, the insulin resistance indexes (HOMA-IR) were figured out. Compared with model group, the FBG and TG level in rats of high dose group of MDG-1 could were decreased with significantly, and the levels of INS and HOMA-IR were decreased, HDL was increased. The levels of TNF-α, CRP in the serum were significantly down-regulated in high dose group of MDG-1. Polysaccharide MDG-1 from Ophiopogon japonicus can decrease glucose, blood lipid and improve insulin resistance in T2DM rats by decreasing the levels of inflammatory factors of TNF-α and CRP.

1. Introduction

Ophiopogon japonicus is a perennial evergreen grass plant of the genus Liliaceae. It is sweet, slightly bitter, slightly cold, and return to heart, lung, stomach meridian. It has the effects of nourishing Yin and moistening the lungs, improving the stomach and promoting fluid, clearing the heart and removing annoyance[1]. Which clinical mainly be used in dry cough, laryngopharyngeal pain, dry mouth injury, internal heat and thirst, intestinal dry constipation and other diseases. The research results showed that ophiopogon japonicus contains many types of active ingredients which include polysaccharides, saponins, flavonoids and volatile oils, and so on. For the past few years, researchers found that ophiopogon japonicus alcohol extract, ophiopogon japonicus water extract and polysaccharide whom could reduce the level of blood glucose of diabetic model animals, to alleviate and reduce insulin resistance in diabetic patients and improve the level of serum insulin[2].

Polysaccharide MDG-1 is a kind of β-D-fructan with a uniform molecular weight which be separation and purification from polysaccharides of ophiopogon, the molecular mass of phase pair is about 5,000[3]. Previous studies have shown that MDG-1 has some effects of reduce the level of blood glucose and improve insulin resistance, but its mechanism is still unclear.

The purpose of study was to observe the effects of Polysaccharide MDG-1 on blood glucose, blood lipids, insulin resistance and inflammatory factors of serum in diabetic rats whom be induced by injection of STZ and feed high-fat and high-sugar diet, it provides theoretical basis for further exploring the hypoglycemic mechanism of MDG-1 and developing new hypoglycemic drugs.

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2. Materials and methods

2.1 Animals.

A total of 70 SPF SD rats, half of each are male and female, and their weight were 200 ± 20g, whom were conventional breed in barrier environment, eating and drinking with free state. Water and feed were irradiation and sterilized by 60 Co, 12h/12h dark cycle[4].

2.2 Reagents and instruments.

STZ, Glyburide(2.5mg), MDG-1 (purity of 99%). Four biochemical kits for blood lipid, insulin free kit, C-reactive protein (CRP) and tumor necrosis factor α (TNF-α) ria kit, they were all Takara.

2.3 Modeling, Grouping and administration.

Rats be randomized after suitability feeding for a week, control group rats were 10 whom be fed normal diet, and to be made in the model group rats were 60 whom be fed high-fat and high-sugar diet. The rats were fed four weeks, intraperitoneal injection the STZ for 35mg·kg^{-1} which be used to make the model[5]. STZ contains citric acid and sodium citrate buffer solution (PH4.4) and the ratio was 1:1.32[6]. Rats were not prohibit drinking but fasted for 12h before intraperitoneal injection the STZ, the values of FBG concentration were measured on 96 h after administration. The success criteria of in T2DM rats is the blood glucose level≥11.1 mmol·L^{-1}[7, 8]. After testing, a total of 55 rats were produced model with successfully, the blood sugar of control group rats were normal.

After the models were made with successfully, according to the values of FBG concentration, the rats were randomly divided into diabetic model group, glibenclamide group and Polysaccharide MDG-1 groups of high dose, medium dose and low dose, each group is 10. Each group rats be continued to give high-fat and high-sugar diet for two weeks in order to consolidate the model. Initiation of administration after the model was made with successfully, control group and diabetic model group rats be given equal volume saline with 20ml·kg^{-1} with intragastric gavage, glibenclamide group rats be given with 0.0003g·kg^{-1} with intragastric gavage, Polysaccharide MDG-1 groups of high dose, medium dose and low dose rats be given MDG-1 aqueous solution with 0.4, 0.2, 0.1g·kg^{-1} respectively, intragastric administration once per day and continued for 7 weeks, during this time, the rats of each group no death.

2.4 Draw Materials.

After the last administration, fasting 10h, FBG was determined by blood samples taken from severed tail rats, the rats were decapitated after anesthesia with 10% chloral hydrate in 3ml·kg^{-1} and collected blood, the blood were left at room temperature for 2h, then, were put into the centrifuge with 3500 revolutions per minute and work for 10m, select serum which be preserved in -20℃ temperature for detection and analysis.

2.5 Detection indicators.

FBG and INS were measured with glucometer and test paper, TNF-α and CRP were measured by radioimmunoassay, and TG,TC,HDL-C,LDL-C were measured by automatic biochemical analyzer. Insulin resistance index: HOMA-IR = (FBG×INS)/22.5[9].

2.6 Statistical Analysis.

Using SPSS 17.0 software for statistical analysis of the obtained data, the results of the indicators were expressed by x±s, significance test using the t test and ANOVA. With P<0.05 was considered statistically significant and P<0.01 as statistically significant difference.

3. Results

3.1 Effects of Polysaccharide MDG-1 on FBG, INS and HOMA-IR in rats.

Compared with the control group, FBG increased significantly in the model group rats (P<0.01). Compared with the model group, FBG decreased in the glibenclamide group rats (P<0.05), and

FBG decreased significantly in the high-dose group rats of Polysaccharide MDG-1 (P<0.01). The results indicated that Polysaccharide MDG-1 could reduce blood glucose in rats. Compared with the control group, INS increased significantly in the model group rats (P<0.01). Compared with the model group, INS decreased in both glibenclamide group rats and high-dose group rats of Polysaccharide MDG-1(P<0.05). The results indicated that Polysaccharide MDG-1 could reduce concentration of insulin in rat serum.

Compared with the control group, HOMA-IR increased significantly in the model group rats(P<0.01). Compared with the model group, HOMA-IR decreased in both glibenclamide group rats and high-dose group rats of Polysaccharide MDG-1, the results showed that Polysaccharide MDG-1 could alleviate insulin resistance. Although both the Polysaccharide MDG-1 of medium dose and low dose groups had some effects on FBG, INS and HOMA-IR, the difference was not statistically significant. Shown in table 1.

Table 1 Effects of Polysaccharide MDG-1 on FBG, INS, HOMA-IR in rats($\bar{x}\pm s$)

Groups	n	Dose/g (mL)·kg^{-1}	FBG(mmol·L^{-1})	INS(mU·L^{-1})	OMA-IR
Control group	10	20	4.45±0.62	57.50±16.04	13.68±2.18
Model group	10	20	23.14±2.08[a]	145.21±22.57[a]	148.56±31.33[a]
Glibenclamide group	10	0.0003	14.02±2.16[b]	122.49±20.11[b]	119.50±22.27[b]
Polysaccharide	10	0.4	12.32±2.10[c]	109.58±20.53[b]	108.25±21.45[b]
MDG-1 group	10	0.2	19.50±1.98	135.64±18.05	134.71±23.18
	10	0.1	19.66±2.07	140.22±21.18	141.90±24.32

Note: compared with the control group, [a]P<0.01; compared with the model group, [b]P<0.05, [c]P<0.01

3.2 Effects of Polysaccharide MDG-1 on serum lipid level in rats.

Compared with the control group, the serum CHOL content and TG level increased significantly in the model group rats(P<0.01). Compared with the model group, TG level decreased (significantly) in the glibenclamide group rats and high-dose group rats of Polysaccharide MDG-1(P<0.05, P<0.01), respectively. Compared with the control group, HDL-C level decreased significantly in the model group rats(P<0.01). Compared with the model group, HDL-C level increased in the glibenclamide group rats and high-dose group rats of Polysaccharide MDG-1(P<0.05). Compared with the control group, LDL-C level increased significantly in the model group rats(P<0.01). Shown in table 2.

Table 2 Effects of Polysaccharide MDG-1 on serum lipid level in rats ($\bar{x}\pm s$)(mmol·L^{-1})

Groups	n	Dose/g (mL)·kg^{-1}	CHOL	TG	HDL-C	LDL-C
Control group	10	20	1.57±0.21	0.66±0.12	0.69±0.03	0.62±0.10
Model group	10	20	12.44±2.24[a]	4.53±1.25[a]	0.39±0.10[a]	8.16±2.05[a]
Glibenclamide group	10	0.0003	8.13±2.50	2.48±0.70[b]	0.60±0.11[b]	7.52±2.11
Polysaccharide	10	0.4	8.26±2.37	2.07±0.81[c]	0.61±0.12[b]	6.96±2.21
MDG-1 group	10	0.2	10.47±2.18	3.66±1.04	0.42±0.10	7.50±2.48
	10	0.1	11.39±3.01	3.95±1.28	0.40±0.14	7.61±2.37

Note: compared with the control group, [a]P<0.01; compared with the model group, [b]P<0.05, [c]P<0.01

3.3 Effects of Polysaccharide MDG-1 on TNF-α and CRP in rats.

Compared with the control group, the serum TNF-α level increased significantly in the model group rats(P<0.01).Compared with the model group, TNF-α level decreased (significantly) in the glibenclamide group rats and high-dose group rats of Polysaccharide MDG-1(P<0.05,P<0.01), respectively. The results showed that Polysaccharide MDG-1 could reduce the content of TNF-α in serum.

Compared with the control group, the serum CRP level increased significantly in the model group rats(P<0.01). Compared with the model group, CRP level both decreased significantly in the glibenclamide group rats and high-dose group rats of Polysaccharide MDG-1(P<0.01). Compared

with the model group, CRP level decreased in the medium-dose group rats of Polysaccharide MDG-1(P<0.05). The results showed that Polysaccharide MDG-1 could reduce the content of CRP in serum. Shown in table 3.

Table 3 Effects of Polysaccharide MDG-1 on TNF-α and CRP in rats ($\bar{x}\pm s$)(ng·mL^{-1})

Groups	n	Dose/g (mL)·kg $^{-1}$	TNF-α	CRP
Control group	10	20	8.54±2.18	1.12±0.15
Model group	10	20	15.09±2.25[a]	2.57±0.41[a]
Glibenclamide group	10	0.0003	10.77±2.51[b]	1.37±0.29[c]
Polysaccharide	10	0.4	9.26±2.07[c]	1.41±0.33[c]
MDG-1 group	10	0.2	12.66±3.01	1.65±0.36[b]
	10	0.1	12.97±2.43	1.88 ±0.40

Note: compared with the control group, [a]P<0.01; compared with the model group, [b]P<0.05, [c]P<0.01

4. Discussion

In recent years, with the socio-economic development, population aging or lifestyle changes, the incidence of diabetes mellitus has increased, and it has become a global public health problem. Epidemiological survey results show that T2DM accounts for 90% of diabetic patients[10,11].

The pathogenesis of T2DM is mainly pancreatic β cell dysfunction and insulin resistance, Among them, insulin resistance is one of the main intrinsic driving factors of T2DM occurrence, accompanied by the development of the whole disease. A huge mass research data showed that chronic inflammatory reaction plays a key role in the occurrence and development of insulin resistance[12]. Therefore, anti-inflammatory therapy can effectively improve the abnormal metabolism of glucose and lipid in diabetic patients and increase insulin sensitivity. At present, the main involved inflammatory factors in insulin resistance of T2DM include tumor necrosis TNF-α, interleukin-6, CRP, serum amyloid A, etc[13-18]. In this study, inflammatory factors TNF-α and CRP were taken as entry points to further explore the mechanism of Polysaccharide MDG-1 for treatment of T2DM.

The results showed that the diabetic rats induced by STZ injection combined with high-fat and high-sugar diet whose had abnormally high blood glucose and blood lipids levels, decreased insulin sensitivity, and the levels of inflammatory factors TNF-α and CRP increased in serum. After adjustment for high doses of Polysaccharide MDG-1, the blood glucose value of diabetic rats decreased, the serum insulin content decreased, the dyslipidemia was improved, increased of insulin sensitivity, decreased of inflammatory factors TNF-α, CRP content in serum. Although the medium dose group of polysaccharide MDG-1 could reduce the content of CRP, it has no significant therapeutic effect on improving the abnormal conditions such as blood glucose and blood lipid.

5. Conclusion

According to the experimental results, it can be inferred that Polysaccharide MDG-1 can effectively improve insulin resistance in type 2 diabetic rats, and play a role in the treatment of diabetes from reducing blood glucose and regulating blood lipid. It is believed that its mechanism of action is anti-inflammatory, which opens up a new way to find new antidiabetic drugs. The mechanism of action at the deeper molecular level needs further exploration and research.

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