

Effects of Marine Drug Propylene Glycol Alginate Sodium Sulfate on Glucose and Lipid Metabolism in Mice

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Abstract: Previous studies have shown that marine drug propylene glycol alginate sodium sulfate (PSS) plays important roles in human diseases. This study mainly explored the effects of PSS on hyperglycemia and hyperlipidemia in diabetic db/db mouse models. The db/db mice were randomly divided into 5 groups (n=12), which were model control group (distilled water), positive control group (metformin), PSS low, medium, and high dose groups (PSS25, PSS50, PSS100) and normal control group (C57/BL, distilled water). The mice in each group had free diet and water for 90 days. During the experiment, food intake was recorded every day and body weight was recorded weekly. In addition, fasting blood glucose and glycosylated hemoglobin levels were measured regularly. Finally, the contents of triglyceride (TG), low-density lipoprotein (LDL-c), high-density lipoprotein (HDL-c) and total cholesterol (TC) in the serum of mice were determined. PSS can significantly reduce fasting blood glucose and glycosylated hemoglobin levels in db/db mice, and improve insulin sensitivity. Moreover, PSS can reduce the fat accumulation of db/db mice and significantly improve the blood lipid level of db/db mice. PSS can significantly improve the symptoms of glucose and lipid metabolism disorders in db/db mice.

Keywords: marine drug, propylene glycol alginate sodium sulfate, glucose and lipid metabolism, mice

1. Introduction

The marine drug propylene glycol alginate sodium sulfate (PSS) is a sulfated polysaccharide compound [1]. Alginic acid was used as the basic raw material to extract alginic acid from kelp. Alginic acid was first degraded to oligospectic alginic acid, and then propylene glycol ester group and sulfate group were introduced by chemical modification. Then the polyanion compounds were composed of β -D-(1,4)-mannuronic acid (M) and α -L-(1,4)-guluronic acid (G) as the basic glycolytic skeleton [2]. PSS has heparin-like structural characteristics and physiological functions. As a polysaccharide drug, PSS generally has a relative molecular weight of 10000~20000, and its distribution width is 1.80. The ratio of mannuronic acid and guluronic acid (M/G) is about 7:3 [3]. As a representative of marine polysaccharide drugs, PSS has extremely important applications in anticoagulation, antithrombosis, lowering blood lipids and improving microcirculation [4].

PSS has been used in clinical practice for more than 20 years, mainly for ischemic cerebrovascular diseases such as cerebral thromboembolism transient ischemic attack and cardiovascular diseases [5]. The total effective rate of PSS in the treatment of cardiovascular and cerebrovascular diseases is 80%-95% [6]. More and more studies have shown that PSS has a good effect on other diseases, such as cancer, diabetes, chronic glomerulonephritis, hepatitis, psoriasis, restless legs syndrome (RLS) [7-9]. In recent years, with the in-depth clinical use of drugs and the discovery of new pharmacodynamics, the reports on the molecular mechanism of PSS have become a hot spot.

However, most of the current research on PSS in diabetes and its complications focus on the monitoring and statistics of clinical data [10]. And there is a lack of systematic preclinical pharmacological research and mechanism exploration of PSS. Therefore, this experiment uses spontaneous diabetes model mice (db/db) to explore the effect of PSS on diabetes. This study will provide a theoretical basis for the rational use of PSS in clinical practice.

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2. Materials and methods

2.1. Experimental reagent

The main experimental reagents in this study include PSS API (Qingdao Zhengda haier Pharmaceutical Co., Ltd.), Metformin Hydrochloride (Met) enteric-coated tablets (Hubei Wushi Pharmaceutical Co., Ltd.), urine albumin determination kit, urine creatinine determination kit, glycation Hemoglobin determination kit, blood tissue TC, TG, HDL-C, LDL-C determination kit (Beijing Kemei Biotechnology Co., Ltd.), BCA protein concentration determination kit (Shanghai Biyuntian Biotechnology Co., Ltd.).

2.2. Experiment equipment

Experimental equipment includes constant temperature water bath (Hangzhou Sijimei Bio-engineering Materials Co., Ltd.), AL-204 electronic analytical balance (Shanghai Mettler-Toledo Instruments Co., Ltd.), Microplate Reader Rainbow (made in Austria); MolliQII pure Water meter (Millipore).

2.3. Experimental animal

SPF male db/db mice (25±2g) and SPF male C57/BL mice (23±2g) were purchased from the Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China). During the experiment, all mice were fed with ordinary feed. And they were free to eat and drink. The environmental conditions are as follows: temperature 20~26°C, relative humidity 40%~70%, light and dark alternately for 12h, keep good ventilation.

2.4. Group administration of experimental animals

db/db mice were randomly divided into model control group, positive control group, PSS low, medium, and high dose groups according to their body weight (n=12). C57/BL mice (n=12) were taken as normal control group. The animals in the above groups were given intragastric administration respectively. The normal control group and the model control group were given distilled water 20 mL/kg/d, and the positive control group was given metformin hydrochloride enteric-coated tablets 225 mg/kg/d. PSS groups (low, medium and high) were given PSS solution, and the doses were 25, 50, 100 mg/kg/d, respectively. The dose volume was 20 mL/kg/d, and the dose was continuous for 90 days.

3. Results and discussions

3.1. Statistical analysis

Data analysis was performed using SPSS 17.0 software. Data were shown as mean ± SD. Independent-samples T test and One-Way ANOVA were used to analyze the difference between groups. Differences were considered statistically significant at $P < 0.05$.

3.2. Results

3.2.1. The effect of PSS on diet and body weight of db/db mice

As shown in Table 1, the increased weight of mice in the model group, PSS group, and positive control group was significantly different from that of the blank group ($P < 0.01$). After intragastric administration of different doses of PSS, the body weight of the mice was decreased. Among them, the mice in the PSS100 group had the largest weight loss, which was significantly different from the model group ($P < 0.01$). Although the effect of the positive drug metformin on the body weight of mice was not obvious, the food intake was significantly lower than that of the PSS100 group ($P < 0.05$). The results show that PSS can significantly reduce the weight of mice without affecting their appetite, and has a certain weight loss effect. Therefore, PSS has shown a tendency to be superior to metformin in the treatment of diabetes.

**Table 1.** The effect of PSS on diet and body weight of db/db mice

Group	Body weight (g)		Average daily intake (g)
	Begin	90d	
C57/BL	23.6 ± 0.38**	30.2 ± 0.44**	4.78 ± 0.26**
db/db	31.2 ± 0.87	48.7 ± 1.2	8.81 ± 0.18
PSS25mg	31.5 ± 1.5	45.1 ± 1.4	8.21 ± 0.2
PSS50mg	32.2 ± 1.1	44.8 ± 1.6	8.14 ± 0.3
PSS100mg	32.5 ± 1.3	43.2 ± 1.3**#	7.84 ± 0.33#
Metformin	32.3 ± 1.4	48.1 ± 1.1	6.82 ± 0.21**

Compared with db/db group, *P<0.05, **P<0.01

Compared with the Metformin control group, #P<0.05

3.2.2. The effect of PSS on fasting blood glucose and glycosylated hemoglobin in db/db mice

At the beginning of the experiment, the fasting blood glucose levels of the mice in the model group, the administration group, and the Metformin control group were increased (Table 2). Compared with the blank group, a significant difference was found ($P < 0.01$, Table 2), indicating that the model was successful. After 90 days of administration, the PSS administration group had lower fasting blood glucose levels compared with the model group. Especially when the PSS dose reached 100 mg/kg, the fasting blood glucose level was significantly different from the model group ($P < 0.05$, Table 2). However, there was no significant difference from the Metformin group. After 90 days, compared with the blank group, the HbA1C levels of the model group, the administration group, and the Metformin control group were significantly increased. However, the PSS administration group can reduce the level of HbA1C in model mice (Table 2). And the level of HbA1C shows a certain dose tolerance of PSS. When the PSS dose reaches 100 mg/kg, the treatment effect of PSS is comparable to Metformin. The results show that PSS has a certain effect on lowering blood sugar.

Table 2. The effect of PSS on fasting blood glucose and glycosylated hemoglobin in db/db mice

Group	HbA1C (90d, nmol/L)	Fasting blood glucose (mmol/L)	
		Begin	90d
C57/BL	190.2 ± 8.95**	4.67 ± 0.12**	5.01 ± 0.19**
db/db	1228.3 ± 49.95	8.16 ± 0.38	25.78 ± 1.12
PSS25mg	1003.6 ± 54.8**#	8.30 ± 0.41	23.1 ± 0.65
PSS50mg	991.2 ± 56.62**#	8.42 ± 0.31	23.4 ± 1.38
PSS100mg	872.5 ± 52.3**	8.32 ± 0.21	21.5 ± 0.87*
Metformin	809.3 ± 30.1**	8.08 ± 0.22	19.1 ± 1.72**

Compared with db/db group, *P<0.05, **P<0.01

Compared with the Metformin control group, #P<0.05

3.2.3. The effect of PSS on insulin sensitivity of db/db mice

The blood glucose level of the mice in each group began to drop sharply 30 min after a one-time injection of 1U/kg of insulin (Table 3). After 60 min, the blood glucose level of the PSS group was significantly lower than that of the model group, and after 120 min it tended to increase slowly. When the PSS dose reaches 100 mg/kg, insulin sensitivity can significantly increase, which is significantly different from the metformin group ($P < 0.05$, Table 3). The results show that PSS100 is better than metformin in improving insulin resistance with long-term medication.

Table 3. The effect of PSS on insulin sensitivity of db/db mice

ITT (1U/kg)	Glucose level (nmol/L)					
	0 min	30 min	60 min	90 min	120 min	150 min
db/db	27.1 ± 1.5	25.8 ± 1.2	20.8 ± 1.6	17.8 ± 1.8	16.9 ± 1.1	17.5 ± 1.2
PSS100mg	26.3 ± 0.55	24.5 ± 0.73	18.5 ± 1.3*	13.2 ± 1.7**#	12.4 ± 1.1**#	12.8 ± 0.7**#
Metformin	24.5 ± 2.9	23.1 ± 2.1	19.5 ± 3.2	16.5 ± 2.8	15.2 ± 2.3	16.4 ± 3.0

Compared with db/db group, *P<0.05, **P<0.01

Compared with the Metformin control group, #P<0.05

3.2.4. The effect of PSS on the changes of blood lipids in db/db mice

As shown in Table 4, PSS (100 mg/kg) can significantly reduce triglyceride (TG) levels, cholesterol (TC) levels, and low-density lipoprotein (LDL-c) levels, and increase high-density lipoprotein (HDL-c) level. And the effect of PSS is better than that of metformin. The results show that PSS has a better effect of regulating blood lipids.

Table 4 The effect of PSS on the changes of blood lipids in db/db mice

Group	TC (mM)	TG (mg/dl)	LDL-c (mM)	HDL-c (mM)
C57/BL	2.01 ± 0.16***	12.6 ± 0.17*	0.43 ± 0.03*	0.76 ± 0.02***
db/db	2.76 ± 0.12	13.18 ± 0.15	0.55 ± 0.02	1.15 ± 0.03
PSS25mg	2.46 ± 0.07#	12.98 ± 0.10	0.51 ± 0.02	1.09 ± 0.04
PSS50mg	2.47 ± 0.17	13.02 ± 0.15	0.52 ± 0.03	1.12 ± 0.03
PSS100mg	2.35 ± 0.07**	12.92 ± 0.11*	0.52 ± 0.04*	1.14 ± 0.02
Metformin	2.64 ± 0.21	13.14 ± 0.24	0.51 ± 0.05	1.10 ± 0.04

Compared with db/db group, *P<0.05, **P<0.01

Compared with the Metformin control group, #P<0.05

3.3. Discussions

In recent years, due to the increasing aging of the population and the influence of unhealthy lifestyles, the incidence of diabetes has increased [11]. Complications caused by long-term diabetic metabolism disorders are also increasing, such as diabetic heart disease (diabetic hypertension, hyperlipidemia, coronary heart disease) and diabetic nephropathy (kidney failure, uremia) [12-13]. These complications have become the main reason for the decline in the quality of life and death of diabetic patients. So far, the clinically used drugs for the treatment of diabetes mainly include biguanides, sulfonylureas, thiazolidinediones, glucagon-like peptide analogs 1, dipeptidyl peptidase IV inhibitors, and α -glycosidase inhibitors [14-15]. But these drugs often have different side effects. Individual drugs may also experience secondary failures, which may even lead to hypoglycemia and liver and kidney diseases [16]. Therefore, there is an urgent need to develop highly effective and low-toxic anti-diabetic drugs.

PSS is a semi-synthetic marine polysaccharide drug with the characteristics of multiple components and multiple targets [17]. PSS first entered the market as an anticoagulant drug. After years of clinical research, it was found that PSS also has many other biological activities [18]. Therefore, it is necessary to further develop and explore the application of PSS in human diseases. PSS has been reported to have a good effect on reducing blood sugar and lipids in a rat model of diabetes induced by streptozotocin [19]. However, the diabetes model induced by streptozotocin is unstable and is affected by many factors, such as the instability of streptozotocin, the control of the injection dose and the number of injections, animal diet, and body weight [20]. Therefore, the international community now advocates the use of spontaneous db/db mouse model.

In order to explore the effects of PSS on diabetes and its complications, this research used a spontaneous diabetes db/db mouse model. The results of the study showed that PSS (100 mg/kg) could effectively reduce the fasting blood glucose level and glycosylated hemoglobin level of db/db mice. Moreover, PSS can reduce the resistance of diabetic mice to insulin and enhance insulin sensitivity. In addition, PSS can also significantly reduce the body weight of model mice without affecting the diet, and improve the blood lipid metabolism of diabetic mice. The efficacy of PSS is equivalent to that of the positive drug metformin for diabetic mice. This study will provide new strong evidence for that PSS can improve diabetes.

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