Repeated oral treatment with polysaccharide sulfate reduces insulin resistance and dyslipidemia in diabetic dyslipidemic rat model

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Abstract: Polysaccharide sulfate (PSS) is a new type of antiatherosclerotic medicine for its effects of anticoagulation, anti-thrombosis and modulation of dyslipidemia. However, it is still uncertain whether PSS could modulate the diabetic dyslipidemia or not. Here, the rat model of diabetic dyslipidemia was developed and the effects of PSS on glucose and lipid levels were investigated in this animal model. Wistar rats were iv injected with streptozotocin 20 mg·kg^{-1} after feeding with high fat diet for one and a half month. Then, rats received orally PSS (30, 90, and 180 mg·kg^{-1}) for 1 month. After oral treatment with PSS (90 and 180 mg·kg^{-1}) for 1 month, the levels of triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) were significantly reduced and the level of high density lipoprotein-cholesterol (HDL-C) increased, compared with diabetic control rats. Moreover, PSS (30, 90, and 180 mg·kg^{-1}) had a tendency to reduce glucose and insulin levels, and significantly increased insulin sensitivity index. Our results suggest that PSS could improve insulin sensitivity and relieve dyslipidemia in diabetic dyslipidemic rats.

Key words: polysaccharide sulfate; experimental diabetes mellitus; streptozotocin; dyslipidemia
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藻酸双酯钠改善模型大鼠的胰岛素抵抗和血脂异常

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摘要: 藻酸双酯钠具有抗凝、抗血栓以及调节血脂等作用, 是一种新型的抗动脉粥样硬化药物。但它对糖尿病伴脂代谢异常血症的治疗作用还没有明确。本研究建立糖尿病伴脂代谢异常血症大鼠模型, 初步探讨藻酸双酯钠对其血糖和血脂的调节作用。Wistar大鼠高脂饲料喂养 1.5个月后尾静脉注射链脲佐菌素。建模成功后, 灌胃给予藻酸双酯钠 1个月, 检测血中血糖、胰岛素和脂质浓度。结果显示, 藻酸双酯钠治疗组与糖尿病对照组相比, 血中甘油三酯、总胆固醇和低密度脂蛋白明显降低, 高密度脂蛋白显著升高, 藻酸双酯钠治疗后血糖和胰岛素浓度有所下降, 而胰岛素敏感指数比糖尿病对照组明显提高。本研究提示, 藻酸双酯钠可以改善糖尿病伴脂代谢异常血症大鼠血脂异常, 并提高胰岛素敏感性。

关键词: 藻酸双酯钠; 实验性糖尿病; 链脲佐菌素; 脂代谢异常血症

As a new type of antiatherosclerotic medicine, polysaccharide sulfate (PSS) possesses the effects of anticoagulation, anti-thrombosis and modulation of dyslipidemia; hence it has been used to prevent and treat ischemic cerebral vascular disease and cardiovascular disease[1-4]. Recently, a few clinic studies have reported that PSS might improve metabolic disorder on type 2 diabetic patients. It is therefore raised the

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question whether PSS can modulate the diabetic dyslipidemia or not. According to the reports fat-fed, streptozotocin-treated rats provide a novel animal model for type 2 diabetes, which is fit for dyslipidemia and suitable for pharmaceutical research[5,6]. In order to understand the mechanisms of PSS for treatment of diabetes, here we investigated the effects of PSS on glucose and lipid levels using diabetic dyslipidemic rats induced by streptozotocin and high fat diet.

Materials and methods

Drug preparation Polysaccharide sulfate powder (Haier Qingdao Third Pharmaceutical Factory, Shandong, China), metformin hydrochloride tables (Baiyunshan Tangmingdongdai Pharmaceuticals Co. Ltd., China), and lovastatin capsules (Heilongjiang Zhaodonghuafu Pharmaceuticals Co. Ltd., China) were prepared by dissolving them separately in saline.

Animal modeling, grouping and treatment Sixty-seven healthy male Wistar rats (Grade II, certificate No. 2003-0019) were obtained from Experimental Animal Center of China Medical University, weighing (150 ± 15) g. The rats were exposed to 12/12-hour light/dark cycle and had free access to food and water. Sixty rats were randomly selected and fed with the high fat chow for one and a half month. Then rats were intravenously injected with 20 mg• kg^{-1} of streptozotocin (STZ, Sigma Chemical Co., MO, USA. Lot. S0130) after an overnight fast.

Two weeks after the injection, 42 fat-fed, streptozotocin-induced diabetic rats determined with fasting blood glucose were randomly divided into six groups as the following: diabetic control group (n = 7), ig saline (10 mL• kg^{-1}); low dose PSS group (n = 7), ig PSS (30 mg• kg^{-1}• d^{-1}); middle dose PSS group (n = 7), ig PSS (90 mg• kg^{-1}• d^{-1}); high dose PSS group (n = 7), ig PSS (180 mg• kg^{-1}• d^{-1}); metformin hydrochloride group (n = 7), ig metformin (200 mg• kg^{-1}• d^{-1}); lovastatin group (n = 7), ig lovastatin (4 mg• kg^{-1}• d^{-1}). Drugs were given between 13∶30 - 14∶30 everyday for 1 month. Seven control rats not injected with STZ were taken as normal control group. All of groups were fed with the conventional chow.

The high fat chow included 50% conventional chow, 30% fat oil, 18% sucrose, 1% cholesterol (Beijing Aoboxing Bio-technology Co. Ltd., China), 0.2% sodium deoxycholate (Beijing Aoboxing Bio-technology Co. Ltd., China).

The diabetic rats were diagnosed according to the modified diabetes diagnosis standard suggested by the America Diabetes Association for human: fast blood glucose (FBG) > 7.0 mmol• L^{-1}.

Biochemical analysis Blood glucose was determined by glucosemetry ONE TOUCH II (LIFESCAN Co. Ltd.). Tail vein blood was sampled for glucose determination before and after treatment with drugs.

Triglycerides (TG) and total cholesterol (TC) were determined by enzyme end-point method, low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) were determined by method of elimination. All of them were determined with Hitachi 7600-Automatic Analyzer Report with commercial kits (Nippon First Pharmaceuticals Co. Ltd.) before and after treatment with drugs. Blood samples were collected from fossa orbitalis plexus venous after an overnight fasting. After separation of blood, an aliquot of serum was taken for measurement of lipid levels. The remainder was for insulin determination.

Serum insulin was measured by radioimmunoassay using insulin reagent kit (China Institute of Atomic Energy, China) at the end of treatment. Insulin sensitivity index was calculated as following:

$$ISI = \ln\left(\frac{1}{[\text{insulin}] \times [\text{glucose}]}\right)$$

Statistical analysis All values are expressed as $\bar{x} \pm s$. Statistical analysis was performed using the SPSS 11.5 program. The data were analyzed by one-way ANOVA. Statistical significance was defined as $P < 0.05$.

Results

1 Some parameters of diabetic dyslipidemic rat model

The Wistar rats were fed with the high fat chow for one and a half month, then injected with STZ 20 mg• kg^{-1}. After 2 weeks, the blood glucose in fat-fed, streptozotocin-induced rats was higher than that in normal control group ($P < 0.01$). Moreover, in fat-fed, streptozotocin-induced rats, the levels of TG, TC and LDL-C were higher, and the level of HDL-C was lower, as compared with that of normal control group ($P < 0.05$ or $P < 0.01$). The data indicated that diabetic dyslipidemic rat model was developed. In this rat model, body weight of rats decreased significantly, whereas water intake increased significantly, and food consumption did not change, compared with normal control rats. All of data showed in Table 1.

Table 1 Changes of the diabetic dyslipidemic rats on body weight, food consumption, water intake, triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and fasting blood glucose

Parameter	Normal control	Diabetic control
Body weight/g	273 ±4	225 ±12*
Food consumption/g	25.0 ±0.4	30.2 ±1.6
Water intake/mL	62.5 ±1.0	117 ±6**
TG/mmol·L^{-1}	0.26 ±0.02	0.50 ±0.06*
TC/mmol·L^{-1}	1.55 ±0.11	3.18 ±0.23**
LDL-C/mmol·L^{-1}	0.26 ±0.03	0.69 ±0.04**
HDL-C/mmol·L^{-1}	1.01 ±0.10	0.68 ±0.06**
Fasting blood glucose/mmol·L^{-1}	5.9 ±0.3	14.2 ±2.1**

$n=7$, $\bar{x}\pm s$. * $P<0.05$, ** $P<0.01$ *vs* normal control group

2 Effect of polysaccharide sulfate on body weight, food consumption and water intake in diabetic dyslipidemic rats

Treatment with polysaccharide sulfate for 1 month, the water intake in polysaccharide sulfate (90 and 180 mg·kg^{-1}) groups was lower than that in diabetic control group ($P<0.05$ or $P<0.01$). However, there was no significant difference on body weight and food consumption between drugs-treated group and diabetic control group (Table 2).

3 Effect of polysaccharide sulfate on lipid metabolic parameters

After drug treatment for 1 month, the levels of TG, TC and LDL-C reduced obviously in polysaccharide sulfate (90 and 180 mg·kg^{-1}), metformin and lovastatin groups compared with those in diabetic control group ($P<0.01$), whereas the level of HDL-C elevated significantly ($P<0.05$ or $P<0.01$). All of data showed in Table 3.

Table 2 Effect of polysaccharide sulfate (PSS) on body weight, food consumption and water intake in diabetic dyslipidemic rats

Group	Dose /mg·kg^{-1}	Body weight /g	Food consumption/g	Water intake /mL
Diabetic control		262 ±14	42.8 ±2.3	169 ±9
PSS	30	298 ±19	42.8 ±2.8	129 ±8
	90	294 ±14	47.1 ±2.2	124 ±6*
	180	289 ±21	38.4 ±2.8	93 ±7**
Metformin	200	286 ±15	44.0 ±2.2	106 ±5**
Lovastatin	4	285 ±9	46.0 ±1.5	138 ±4

$n=7$, $\bar{x}\pm s$. * $P<0.05$, ** $P<0.01$ *vs* diabetic control group

4 Effect of polysaccharide sulfate on glucose metabolic parameters

After drug treatment for 1 month, insulin sensitivity index was significantly heightened by polysaccharide sulfate ($P<0.01$) and metformin ($P<0.01$). Blood glucose was lowered in metformin group ($P<0.05$), but not in polysaccharide sulfate group. In contrast, both PSS and metformin groups decreased serum insulin levels, but significance was only observed in PSS (90 and 180 mg·kg^{-1}) groups ($P<0.01$, Table 4).

Table 3 Effect of polysaccharide sulfate (PSS) on triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C)

Group	Dose /mg·kg^{-1}	TG/mmol·L^{-1}	TC/mmol·L^{-1}	LDL-C/mmol·L^{-1}	HDL-C/mmol·L^{-1}
Diabetic control		0.56 ±0.06	2.79 ±0.16	0.70 ±0.04	0.65 ±0.09
PSS	30	0.46 ±0.07	1.92 ±0.14**	0.52 ±0.10**	0.86 ±0.08*
	90	0.32 ±0.04**	1.62 ±0.08**	0.41 ±0.02**	0.86 ±0.04*
	180	0.26 ±0.05**	1.59 ±0.10**	0.36 ±0.04**	0.95 ±0.06**
Metformin	200	0.34 ±0.06**	1.95 ±0.10**	0.46 ±0.03**	0.90 ±0.08*
Lovastatin	4	0.24 ±0.03**	1.50 ±0.08**	0.40 ±0.04**	0.96 ±0.10**

$n=7$, $\bar{x}\pm s$. * $P<0.05$, ** $P<0.01$ *vs* diabetic control group

Table 4 Effect of polysaccharide sulfate (PSS) on fasting blood glucose, fasting blood insulin and insulin sensitivity index

Dose	Dose /mg·kg^{-1}	Fasting blood glucose/mmol·L^{-1}	Fasting blood insulin/mmol·L^{-1}	Insulin sensitivity index
Diabetic control		15.0 ±1.9	23.1 ±3.2	-5.74 ±0.15
PSS	30	10.6 ±2.9	19.3 ±3.0	-5.05 ±0.12**
	90	13.7 ±2.1	12.9 ±2.0**	-5.03 ±0.19**
	180	12.3 ±3.0	12.6 ±1.5**	-4.85 ±0.22**
Metformin	200	8.2 ±0.5*	16.2 ±2.6	-4.82 ±0.14**
Lovastatin	4	14.9 ±2.1	15.8 ±0.7	-5.39 ±0.18

$n=7$, $\bar{x}\pm s$. * $P<0.05$, ** $P<0.01$ *vs* diabetic control group

Discussion

The high fat diet leads to insulin resistance and streptozotocin leads to abnormal insulin secretion[7-10]. By combining high fat diet and STZ administration, Zhang FL et al[11] developed a rat model of type 2 diabetic mellitus. In the present study, we initiated our efforts to establish a rat model which became insulin resistant after a given period of high fat diet, and then added a relative low dose of STZ (20 mg• kg^{-1}) administration. About 70% of experiment rats were developed into diabetic dyslipidemic rat model, which was characterized by hyperglycemia and dyslipidemia and simulated the natural history and metabolic characteristics of patients with type 2 diabetes[12]. Unexpectedly, the diabetic dyslipidemic rats decreased significantly on body weight compared with normal control rats, although there was no difference on their food consumption. The pattern of weight change in this rat model is not yet well understood and it may be not just attributed to appetite.

With this model, we investigated the effects of polysaccharide sulfate on blood glucose and lipid levels. It was demonstrated that polysaccharide sulfate did not influence body weight of the diabetic dyslipidemic rats. As we know, diabetic dyslipidemia is characterized by low concentration of HDL-C and high concentrations of LDL-C combined with hypertriglycemidemia[13-15]. In the present study, polysaccharide sulfate decreased the levels of serum TG, TC and LDL-C, and increased serum HDL-C levels. In addition, these effects in polysaccharide sulfate 90 and 180 mg• kg^{-1} • d^{-1} groups were found to be better than that in the dose of 30 mg• kg^{-1} • d^{-1}. The result is continence with several clinic observations[16].

Insulin sensitivity index can be assessed by many methods. Eu-glycemic clamp method is most accurate one, but it is difficult and expensive. We have used an easy but recognized method proposed by Li GW et al[17]. Polysaccharide sulfate has improved insulin sensitivity index, although the blood glucose level has no significant change compared with those in diabetic control group. Polysaccharide sulfate might possess facilitating insulin secretion property, or reducing insulin resistance of the peripheral tissues. Or this effect may be a consequence of relieving dyslipidemia.

Although the exact mechanisms remain uncertain, we found that polysaccharide sulfate could improve insulin sensitivity and relieve dyslipidemia in diabetic dyslipidemic rats, and it should assist physicians in finding more suitable drugs for treating type 2 diabetic patients with dyslipidemia.

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