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Changes of adipocytokine expression after diabetic rats received sitagliptin and the molecular mechanism

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ABSTRACT

Objective: To study the effect of sitagliptin on adipocytokines expression in diabetic rats and its molecular mechanism.
Methods: Male SD rats were chosen and randomly divided into NC group, T2DM group, SP group and SP + LY group. NC group received conventional breeding, T2DM group, SP group and SP + LY group received intraperitoneal injection of streptozotocin after 12 weeks of high-fat diet to establish diabetes animal model, SP group received sitagliptin intervention and SP + LY group received sitagliptin combined with PI3K inhibitor LY294002 intervention. Six weeks after the intervention, serum was collected to determine the levels of biochemical indexes and adipocytokines, and visceral adipose tissue was collected to determine expression levels of adipocytokines.
Results: Serum TC, TG, LDL-C, FBG, FINS, Leptin and Chemerin levels as well as HOMA-IR of T2DM group were higher than those of NC group, and HDL-C, Adiponectin and Omentin-1 levels were significantly lower than those of NC group; serum TC, TG, LDL-C, FBG, FINS, Leptin and Chemerin levels as well as HOMA-IR of SP group were lower than those of T2DM group, and HDL-C, Adiponectin and Omentin-1 levels were significantly higher than those of T2DM group; Leptin and Chemerin levels in serum and visceral adipose tissue of SP + LY group were higher than those of SP group while Adiponectin and Omentin-1 levels were significantly lower than those of SP group.
Conclusion: Sitagliptin can regulate the expression of adipocytokines in adipose tissue of diabetic rats through PI3K-AKT pathway.

1. Introduction

Type 2 diabetes mellitus (T2DM) is an endocrine disease characterized by insulin resistance and relatively insufficient insulin secretion, is with rising morbidity, and causes adverse effect on both life health and quality of life of the patients. Adipocytokines are a type of cytokines synthesized and secreted by adipocytes, include Adiponectin, Leptin, (Omentin-1), Chemerin, *etc.*, and have regulating effect on inflammation, insulin sensitivity, endothelial function, *etc* [1,2]. There are abnormal glucolipid metabolism and abnormal abdominal visceral adipose tissue accumulation in T2DM patients, and the adipocytokines synthesized by visceral adipose tissue will change, resulting in a variety of diabetic complications [3–5]. Sitagliptin is an oral hypoglycemic drug used in treatment of T2DM patients in recent years, belongs to the dipeptidyl peptidase-4 (DPP-4) inhibitor, and can inhibit glucagon-like peptide (GLP-1) degradation, regulate insulin sensitivity and lower blood glucose [6,7]. At present, the regulating effect of sitagliptin on adipocytokines in patients with T2DM is unclear. In the following study, the diabetic rat models were established and the changes of adipocytokines after sitagliptin treatment were analyzed.

2. Materials and methods

2.1. Experimental materials

Experimental animals were 50 male SD rats with 6–8 weeks of age and body mass of 160–220 g, they were purchased from

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the experimental animal center of Zhejiang University, and license No. was SCXK 2008-0016, the animal study was approved by the hospital ethics committee; streptozotocin and PI3K inhibitor LY294002 were from Sigma Company, enzyme-linked immunosorbent assay kits were from Elabscience Company, and RNA extraction kits, the first strand of cDNA synthesis kits and fluorescence quantitative PCR kits were from Beijing Tiangen Biochemical Company. OneTouch Ultra for Johnson & Johnson Company, microplate reader was from the Bio-rad Company, and fully automatic biochemical analyzer was from Switzerland Roche Company.

2.2. Experimental methods

2.2.1. Animal grouping and model establishing methods

Experimental animals were randomly divided into NC group, T2DM group, SP group and SP + LY group. NC group (n = 8) received conventional breeding, the remaining 42 were used for T2DM model establishment, and the method was as follows: they received high-fat diet for 12 weeks, and then were given intraperitoneal injection of 35 mg/kg streptozotocin, streptozotocin was temporarily configured with pH = 4.5 0.1 mmol/L sodium citrate buffer before injection, blood was collected via caudal vein 7 days after injection, rats with fasting glucose > 11.1 mmol/L were considered as successfully established, 5 were dead, 7 were with substandard fasting glucose, and only 30 were successfully established and randomly divided into T2DM group, SP group and SP + LY group, 10 in each group.

2.2.2. Intervention methods

T2DM group received intragastric administration of hydroxymethyl amylase, 2 mL/time, 1 time/day, and then continued to receive normal diet; SP group received intragastric administration of sitagliptin, 10 mg/(kg·d), the sitagliptin was dissolved in 2 ml hydroxymethyl amylase, and after intervention, they continued to receive normal diet; SP + LY group received intragastric administration of sitagliptin with the same method as that of SP group and received subcutaneous injection of PI3K inhibitor LY294002, 50 μg/(kg·d), LY294002 was dissolved in 1 mL saline, and after intervention, they continued to receive normal diet.

2.2.3. Sample collecting methods

Six weeks after intervention, four groups of rats were fasting for 12 h and then was sacrificed by cervical dislocation, blood samples were collected, let stand for 15 min at room temperature and then centrifuged in the centrifugal machine for 10 min at 3000 r/min, and serum was separated and saved at −80 °C; abdominal cavity of rats was opened after execution, abdominal visceral adipose tissue was collected, frozen with liquid nitrogen and then saved at −80 °C.

2.2.4. Index detecting methods

Serum specimen were collected, automatic biochemical analyzer was used to determine fasting blood glucose (FBG), fasting blood insulin (FINS), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) content, and the steady-state model was used to calculate the insulin resistance index (HOMA-IR) = FINS × FBG/22.5. Enzyme-linked immunosorbent assay kits were used to determine Adiponectin, Leptin, Omentin-1 and Chemerin levels. Abdominal visceral adipose tissue was collected, RNA extraction kits and the first strand of cDNA synthesis kits were used to separate and obtain cDNA samples, then fluorescent quantitative PCR amplification was performed, and the mRNA levels of Adiponectin, Leptin, Omentin-1 and Chemerin were calculated.

2.2.5. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data was by variance analysis, pair-wise comparison was by LSD-t test and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1. Serum biochemical indexes of all groups

Serum biochemical indexes TC, TG, LDL-C, HDL-C, FBG and FINS as well as HOMA-IR of NC group, T2DM group, SP group and SP + LY group were significant different ($P < 0.05$), and pair-wise comparison and analysis showed that: serum TC, TG, LDL-C, FBG and FINS levels as well as HOMA-IR of T2DM group were higher than those of NC group, and HDL-C was significantly lower than that of NC group; serum TC, TG, LDL-C, FBG and FINS levels as well as HOMA-IR of SP group were lower than those of T2DM group, and HDL-C was significantly higher than that of T2DM group; serum TC, TG, LDL-C, FBG and FINS levels as well as HOMA-IR of SP + LY group were higher than those of SP group, and HDL-C was significantly lower than that of SP group (Table 1).

3.2. Effect of sitagliptin intervention on serum levels of adipocytokines in T2DM rats

Serum adipocytokines Adiponectin, Leptin, Omentin-1 and Chemerin levels of NC group, T2DM group and SP group were

Table 1
Serum biochemical indexes of all groups.

Group	n	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	FBG (mmol/L)	FINS (IU/mL)	HOMA-IR
NC group	8	1.74 ± 0.25	0.72 ± 0.10	0.97 ± 0.12	0.82 ± 0.10	4.89 ± 0.74	6.14 ± 0.89	1.62 ± 0.22
T2DM group	10	2.95 ± 0.32△	1.32 ± 0.18△	1.93 ± 0.25△	0.45 ± 0.07△	13.20 ± 1.85△	17.58 ± 2.51△	8.48 ± 1.03△
SP group	10	2.04 ± 0.31▲	0.89 ± 0.11▲	1.21 ± 0.18▲	0.74 ± 0.09▲	5.85 ± 0.71▲	8.39 ± 1.05▲	3.38 ± 0.44▲
SP + LY group	10	2.77 ± 0.35*	1.25 ± 0.22*	1.82 ± 0.26*	0.56 ± 0.07*	12.14 ± 1.88*	14.59 ± 2.35*	7.14 ± 0.91*

△$P < 0.05$ compared with NC group; ▲$P < 0.05$ compared with T2DM group; *$P < 0.05$ compared with SP group.