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Anti-diabetic effect of citrus pectin in diabetic rats and potential mechanism via PI3K/Akt signaling pathway

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ABSTRACT

This study was performed to investigate the anti-diabetic effect of citrus pectin in type 2 diabetic rats and its potential mechanism of action. The results showed that fasting blood glucose levels were significantly decreased after 4 weeks of citrus pectin administration. Citrus pectin improved glucose tolerance, hepatic glycogen content and blood lipid levels (TG, TC, LDL-c and HDL-c) in diabetic rats. Citrus pectin also significantly reduced insulin resistance, which played an important role in the resulting anti-diabetic effect. Moreover, after the pectin treatment, phosphorylated Akt expression was upregulated and GSK3β expression was downregulated, indicating that the potential anti-diabetic mechanism of citrus pectin might occur through regulation of the PI3K/Akt signaling pathway. Together, these results suggested that citrus pectin could ameliorate type 2 diabetes and potentially be used as an adjuvant treatment.

1. Introduction

Diabetes mellitus is one of the most common and serious metabolic diseases worldwide, affecting 382 million people [1]. Type 2 diabetes mellitus (T2DM) is the major type of diabetes, accounting for more than 90% of diabetic cases [2]. T2DM is characterized by insulin resistance, which results in impaired glucose tolerance and hyperglycemia [3]. Although synthetic anti-diabetic agents are widely used to treat diabetes, these agents can cause serious side effects [4]. Therefore, effective, naturally sourced compounds have attracted a great deal of attention for the treatment of diabetes.

In recent years, considerable attention has been focused on the anti-diabetic effects of dietary fiber [5–7]. Pectin is a natural polysaccharide found within the cell walls of most fruits and vegetables. In addition, pectin is a significant source of soluble dietary fiber. Pectin is considered safe for human consumption and is used in both food and pharmaceutical products as a thickener and a gelling agent [8]. Over the years, the physiological functions of pectin have been widely investigated, and anti-diabetic, hypolipidemic and anti-cancer effects have been reported [9–11]. Sánchez showed that apple pectin led to improved insulin resistance in Zucker fatty rats [12]. Furthermore, Palou demonstrated that apple pectin could ameliorate insulin sensitivity in adult rats with metabolic disorders [13]. However, the effect of pectin on blood glucose metabolism in T2DM remains to be elucidated.

Insulin resistance is an important indicator in the diagnosis and treatment of diabetes [3,14]. In insulin resistance, abnormal insulin signaling occurs in the liver and muscles. The PI3K/Akt signaling pathway plays a pivotal role in insulin signal transduction [15], and numerous studies have explored the hypoglycemic mechanisms of different anti-diabetic agents acting through the PI3K/Akt signaling pathway [16–18]. Therefore, due to the importance of this pathway, investigating changes in the protein expression would yield useful insights into the anti-diabetic mechanism of citrus pectin.

The aim of this study was to investigate the anti-diabetic effect of citrus pectin in T2DM rats. The potential mechanism of the anti-diabetic effect of citrus pectin via the PI3K/Akt signaling pathway was also explored, which might provide useful functional insights.

2. Materials and methods

2.1. Materials and chemicals

Citrus pectin was extracted and purified from *Citrus sinensis* Osbeck fruit peels according to previously described methods [19]. Streptozotocin (STZ) was purchased from Sigma (Saint Louis, USA). Metformin was purchased from Jingfeng Pharmaceutical Co. (Beijing, China). TC, TG, HDL-c, LDL-c and glycogen assay kits were purchased from Jiancheng Bioengineering Institute (Nan-

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jing, China). The insulin ELISA kit was purchased from Shanghai Yapu Biological Technology Co. (Shanghai, China). Antibodies to phosphorylated Akt (p-Akt) (Ser473) (Cell Signaling Technology, Danvers, USA) and GSK3β (Proteintech Group, Wuhan, China) were used in the Western blotting assay. Secondary antibodies were purchased from Boster Biological Engineering Co. (Wuhan, China). The other reagents were of analytical grade.

2.2. Animals

Male SD rats (180–200 g) were purchased from Hubei Provincial Center for Disease Control and Prevention (Wuhan, China). The rats were housed under controlled conditions at a temperature of 25 ± 1 °C and a humidity of 55 ± 5% with a 12-h light/dark cycle and free access to food and water. This study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Experimental design

After one week of acclimatization, the rats were randomly divided into two groups. Rats in the normal control group (6 rats) were fed a standard pellet diet. The rats in the experimental group were fed a high-fat diet (10% lard, 10% sucrose, 5% yolk, 1% cholesterol, 0.2% sodium deoxycholate and 73.8% standard pellets) to induce diabetes. After 4 weeks, all rats were fasted overnight with free access to water. Rats in the high-fat diet group were injected intraperitoneally with fresh STZ solution (0.1 mol/L citrate buffer, pH 4.5) at a dose of 35 mg/kg body weight (bw). Rats in the normal control group were injected with citrate buffer. At 72 h after STZ administration, fasting blood glucose (FBG) levels were determined using a glucometer (Sinocare, Changsha, China). The rats with FBG levels over 11.1 mmol/L were considered diabetic rats.

The diabetic rats were then randomly divided into five groups, i.e., the diabetic control group, the metformin group (250 mg/kg bw per day), the low-dose citrus pectin group (500 mg/kg bw per day), the medium-dose citrus pectin group (1000 mg/kg bw per day) and the high-dose citrus pectin group (2000 mg/kg bw per day), with six rats each. All of these rats were fed a high-fat diet. Rats in the three citrus pectin groups were intragastrically administered citrus pectin every day. Rats in the diabetic control and normal control groups were administered vehicle (distilled water), and rats in the metformin group were administered metformin. All rats had free access to food and water for 4 weeks.

2.4. Oral glucose tolerance test (OGTT)

OGTT was performed in the last week of the treatment after the rats were fasted for 12 h. In brief, after pectin or vehicle administration, rats were orally administered with glucose at a dose of 2 g/kg bw. Blood glucose levels were then measured at 0, 30, 60 and 120 min after glucose administration.

2.5. Biochemical analysis

FBG levels in blood samples gathered from the tail vein were measured once a week. After 4 weeks of treatment, the rats were fasted for 12 h with free access to water. Blood samples were collected from the retrobulbar venous plexus and centrifuged (4 °C, 4000 r/min, 10 min). Livers and muscles were immediately removed and stored at −80 °C until analysis. TC, TG, HDL-c, LDL-c, fasting serum insulin (FINS) and glycogen levels in the livers and muscles were measured according to the instructions of the assay kits.

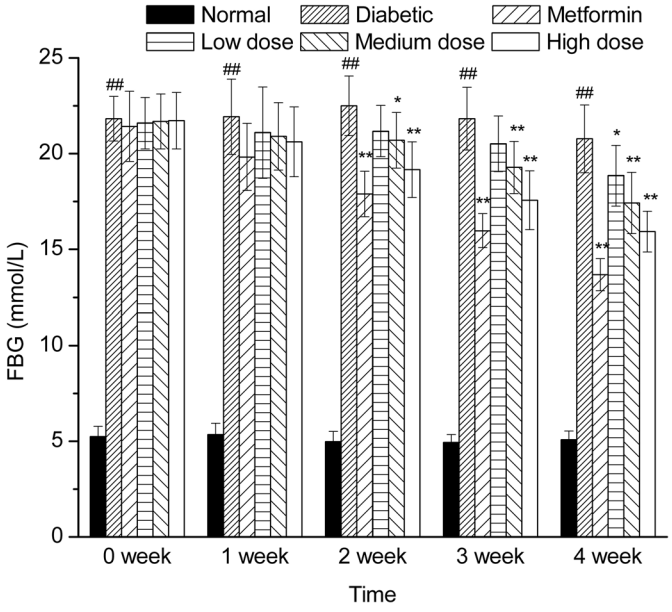

Fig. 1. Effect of citrus pectin on FBG levels in rats. The values are expressed as means ± SD (n = 6). ##P < 0.01, compared with normal control. *P < 0.05, **P < 0.01, compared with diabetic control.

2.6. Insulin resistance

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula to measure the insulin sensitivity of the rats: HOMA-IR = (FBG × FINS)/22.5 [20].

2.7. Western blotting analysis

The hepatic tissues were homogenized in lysis buffer on ice and solubilized for 30 min. After centrifugation, the lysate protein concentrations were measured using a BCA protein assay kit. The proteins were separated electrophoretically using SDS-PAGE and were then transferred to a polyvinylidene difluoride membrane. The membrane was blocked in solution containing 5% skim milk at room temperature for 2 h. Then, the membrane was incubated with primary antibodies against p-Akt, GSK3β or β-actin at 4 °C overnight. After being washed, the membrane was incubated with horseradish peroxidase-conjugated secondary antibodies at 37 °C for 2 h. The protein blots were visualized using ECL reagents and quantified using BandScan software.

2.8. Statistical analysis

The data are expressed as the mean ± SD. Differences between groups were assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test using SPSS 17.0 software. P < 0.05 and P < 0.01 were considered statistically significant.

3. Results

3.1. Effect of citrus pectin on FBG levels

The effect of citrus pectin on FBG levels in diabetic rats is shown in Fig. 1. There was no significant difference in FBG level between the diabetic control and pectin groups at the start of the study (P > 0.05); however, FBG levels in the diabetic control group and citrus pectin groups were higher than those in the normal control group. After 2 weeks of citrus pectin administration, the medium- and high-dose groups showed significantly lower FBG levels com-