Effects of Angelica Keiskei Chalcone on Insulin Resistance of Skeletal Muscle Cells of Type 2 Diabetic Rats*

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ABSTRACT Objective: To investigate the effect of angelica keiskei chalcones (AC) on insulin resistance of skeletal muscle cells of type 2 diabetic rats. Methods: Rats with type 2 diabetes were given 30, 10, 5 mg/ (Kg·bw) AC daily by mouth as high, middle and low dose groups respectively. Sailine was given to the diabetic control group by mouth. Four groups were fed with the diet containing high fat. After four weeks, fasting blood glucose was determined by glucose oxidase method. Insulin was assessed by radio-immunity method. The protein expression levels of Glut1 and Glut4 in skeletal muscle cells were analyzed by immunohistochemistry method. Results: After analyzed by Image-Pro Plus on immunohistochemistry pictures, the expression levels of Glut1 and Glut4 in skeletal muscle cells in high group were 0.054± 0.0064 and 0.063± 0.0139, which were significantly higher than the diabetic control group (P<0.05).The levels of fasting blood glucose and insulin in high dose group were (12.3± 1.64) mmol/L and (25.65± 3.34) μIU/ml , significantly lower than those of the diabetic control group (P<0.05). Conclusion: Angelica keiskei Chalcones may increase the expression levels of Glut1 and Glut4 in skeletal muscle cells, decrease fasting blood glucose and insulin of type 2 diabetic rats and improve their insulin resistance condition.

Key words: Angelica keiskei chalcone; Type 2 diabetes mellitus; Insulin resistance; Glucose transporter
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Introduction

Angelica keiskei chalcone (AC) is a kind of flavonoid compounds. It exists in angelica keiske which is a species of umbelliferae, biennial or perennation and herbaceous plant[1,2]. Studies have shown that angelica keiskei chalcone provided numerous benefits, including suppression of gastric acid secretion, an action as an antitumour agent, an antithrombotic effect, a reduction in blood pressure, suppression of histamine release and promotion of blood circulation[3]. But there is no report on how angelica keiskei chalcone impacts on glucose transporter protein. This study was to evaluate the insulin resistance-improving effect of angelica keiskei chalcone by determining the expression levels of Glut1and Glut4 and the levels of blood glucose and insulin.

1 Materials and Methods

1.1 Materials

1.1.1 Animal Male wistar rats (SPF grade), 170± 20 g weight, were provided by lab center of Shandong Lu kang medicines co., Ltd. (license: SCXK(LU)20090007) . All rats were fed in clean animal house adaptively and grouped after a week. High fat feed and ordinary feed were bought from institute of laboratory animal sciences, CAMS& PUMC.

1.1.2 Experimental sample Angelica keiskei chalcone was offered by Qingdao Hailongda Biochemistry Technology C0., Ltd. Its purity is more than 90%. The standard was purchased from American sigma company.

1.1.3 Reagents and equipments Streptozocin (STZ) was provided by American sigma company. Insulin radioimmunoassay kit was purchased from Beijing North Institute of Biotechnology. Rabbit anti mouse glucose transporter1 polyclonal antibody, rabbit anti mouse glucose transporter4 polyclonal antibody and kit were purchased from Beijing Biosynthesis Biotechnology Co.,LTD. Glucose meter and test papers, microscope, camera were the products of OLYMPUS company.

1.2 Methods

1.2.1 Manufacture type 2 diabetic rats model Experiment rats were fed high fat fodder continuously four weeks, in the fifth week, first, 3th and 5th day, gave STZ 25 mg/(kg·bw), 15 mg/ (kg·bw), 15 mg/(kg·bw)via intraperitoneal injection respectively. After 72 hours, detected the tail blood fasting blood glucose and choise the rats with blood glucose levels > 16.7 mmol/L as successfully models.

1.2.2 Animal groups and treatments Rats with type 2 diabetes were divided into four groups randomly and given 30, 10, 5, 0 mg/ (kg·bw)AC daily by mouth as high, middle and low dose groups

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and diabetic control group respectively. Each group was fed with high fat feed. The same amount of physiological saline were given to the normal control group by mouth and fed with normal feed. After four weeks, injected into peritoneal cavity with 10 % chloral hydrate to anaesthetise. Drew blood from the abdominal aorta and separated serum and took quadriceps femorist, then tested the indicators.

1.3 Detection index

1.3.1 Protein expressions of skeletal muscle Glut Used immunohistochemistry SP method for the determination. Fixed quadriceps femoris with 4 % paraformaldehyde, used paraffin-embedded, made sections, dropped primary antibody, dropped secondary antibody, stained with DAB and stained with hematoxylin. Under highpower microscope, selected visual field randomly, observed the protein expression levels of Glut1 and Glut4, analysed optical density by Image-ProPlus image software, and showed the protein expression levels of Glut with mean optical density(MOD).

1.3.2 Plasma glucose level Fasting blood glucose was determined by glucose oxidase method.Blood was taken from every rat tail separately, droped on test papers. Then red datas displaying on the glucose meter.

1.3.3 The serum insulin level Tested by radioimmunoassay. Drew 0.1 ml serum to the sedimentation tube, then add labelling antibody, incubated at 37℃. After dealing with separation agent and centrifugal separation, examined the radioactive counting in the sedimentation tube. Determined radioactive counting according to log-logit drawing standard curve, then calculated the level of serum insulin.

1.4 Statistical analysis

One-factor analysis of variance(ANOVA) was used for statistical analysis(a=0.05). All analyses were performed by using SPSS 18.0 software.

2 Results

2.1 The protein expression level of Glut1 in skeletal muscle

Glut1 expressing predominantly on the cell membrane were brown yellow granules. The number of Glut1 proteins tested in positive cells were higher than other groups. They were dyed deeply and expressed highly. The mean optical density values in high dose group and the diabetic control group were 0.054± 0.0064 and 0.020± 0.0074 and the difference had statistical significance (P<0.05). MOD values of the protein expression of Glut1 in each group were shown in Table 1.

2.2 The protein expression level of Glut4 in Skeletal muscle

Glut4 expressing in cytoplasm predominantly were brown yellow granules. The number of Glut4 proteins tested in positive cells were higher than other groups. They were dyed deeply and expressed highly. The mean optical density values in high dose group and the diabetic control group were 0.063± 0.0139 and 0.018± 0.0087and the difference had statistical significance(P<0.05). MOD values of the protein expression of Glut4 in each group were shown in Table 1.

Table 1 The protein expressions of Glut1 and Glut4 in skeletal muscle cells in each group

Groups	Rat number	Glut1	Glut4
High dose group	10	0.054± 0.0064 [a]	0.063± 0.0139 [a]
Middle dose group	10	0.031± 0.0122 [ab]	0.036± 0.0140 [ab]
Low dose group	10	0.024± 0.0080 [b]	0.022± 0.0114 [b]
Diabetic control group	10	0.020± 0.0074 [b]	0.018± 0.0087 [b]
Normal control group	10	0.066± 0.0116 [ab]	0.075± 0.0120 [ab]

Notes: a: P<0.05 with diabetic control; b: P<0.05 with high chalcone group; (F Glut1 =45.665, F Glut4 =42.702).

2.3 Fasting plasma glucose and fasting insulin levels

Compared with diabetes control group, fasting plasma glucose and fasting insulin levels in high dose group were reduced, and the difference had statistically significant (P < 0.05). Results were shown in Table 2.

Table 2 The fasting blood glucose and fasting insulin in each group

Groups	Rat number	Blood glucose(mmol/l)	Insulin(μiu/ml)
High dose group	10	12.3± 1.64[a]	25.65± 3.34[a]
Middle dose group	10	17.8± 1.73[b]	45.95± 3.83[b]
Low dose group	10	18.1± 1.96[b]	47.02± 3.16[b]
Diabetic control group	10	18.2± 1.51[b]	48.15± 2.83[b]
Normal control group	10	5.1± 0.96[ab]	15.03 ± 3.43[ab]

Notes: a :P<0.05 with diabetic control; b :P<0.05 with high chalcone group; (F blood glucose =127.227, F insulin = 205.713).

3 Discussion

Insulin resistance is an important manifestation of type 2 diabetes mellitus. In all aspects of insulin resistance, significance of post-insulin receptor defect is very important [4-6]. The peripheral tissues of insulin resistance are skeletal muscle, liver and adipose tissues. The main reason of post-insulin receptor defect is the poor glucose-uptaking ability of these tissues. Improving insulin resistance status of peripheral tissues that is very important for systemic blood glucose control.

Glucose transporter (Glut) is a protein family, which has 14 subtypes, named as Glut1-12, Glut13 (HMIT), Glut14 [7,8]. There are two kinds of glucose transporters which are Glut1and Glut4 in skeletal muscle. They exist in cells and transport glucose from blood to cells. Glut1 exist in most embryonic and adult tissues, which is known to be the most widely distributed glucose transporter and generally transport glucose with other transporters in tissues. In skeletal muscle, Glut1 only transports glucose in basal conditions and provide basic glucose for cells[9,10]. Studies showing that, PI3K and Akt involvement in the effect of insulin on Glut1. Akt with continuing active form can increase the protein expression levels of Glut1, while the up-regulation effect of insulin on Glut1 would be suppressed if Akt were inactivation[11-13]. There are experiments showing that stimulation by the high concentration of sugar reduces protein numbers of Glut1 and glucose absorption[14]. This study showed that angelica keiskei chalcone increased the protein expression of Glut1 in skeletal muscle, so blood glucose was transported into skeletal muscle cells from blood, and then the glycogen synthesis increased and blood sugar concentration reduced.

Glut4 majorly distributed in skeletal muscle cells. After a meal and sport, glucose transport rate of Glut4 is dramatically improved, so that the glucose is rapidly transited into skeletal muscle cells and provides energy for skeletal muscle rapidly [15-18]. Insulin integrates with insulin receptors which are on extraneous coat of cell, then they product a set of signals which lead vesicles containing Glut4 to move to and make fusion with cell membranes[19,20]. Glut4 are translocated to the extraneous coat of cells, and the quantity of Glut4 there increase significantly, so more glucose are transported. Improving protein expression of Glut4 that helps for glucose metabolism and glucose homeostasis. This study showed that angelica keiskei chalcone increased the expression level of Glut4, and then enhance glucose ransportation.

Associated with this study, it can be thought angelica keiskei chalcone was ability to reduce levels of blood glucose and serum insulin of type 2 diabetic rats,and then improved insulin resistance. The mode of action maybe associate with Glut1 and Glut4 which showed high expressions and then increased the blood glucose transport from blood into the cells.

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明日叶查尔酮对2型糖尿病大鼠骨骼肌细胞胰岛素抵抗的干预作用*

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摘要 **目的**:研究明日叶查尔酮对2型糖尿病大鼠骨骼肌胰岛素抵抗的干预作用。**方法**:将2型糖尿病大鼠随机分成四组,高、中、低剂量组分别每日经口灌胃给予明日叶查尔酮30、10和5mg/(kg·bw),糖尿病对照组给予等量生理盐水。各组均以高脂饲料喂养。四周后采用葡萄糖氧化酶法检测空腹血糖;放射免疫法检测血清胰岛素含量;免疫组化法检测葡萄糖转运体1和葡萄糖转运体4蛋白表达水平。**结果**:经图像分析,高剂量组骨骼肌细胞中葡萄糖转运体1和葡萄糖转运体4蛋白表达平均光密度值分别为0.054± 0.0064和0.063± 0.0139,均较糖尿病对照组显著性升高(P<0.05)。高剂量组空腹血糖和胰岛素水平分别为(12.3± 1.64) mmol/L和(25.65± 3.34) (μIU/mL),均较糖尿病对照组显著性降低(P<0.05)。**结论**:明日叶查尔酮可增加2型糖尿病大鼠骨骼肌葡萄糖转运体1和葡萄糖转运体4蛋白表达水平,降低空腹血糖和胰岛素水平,改善胰岛素抵抗状况。

关键词:明日叶查尔酮;2型糖尿病;胰岛素抵抗;葡萄糖转运体

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