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Preventive effects of ophiopogon−polysaccharide on apiponectin in gestational diabetes mellitus rat

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ABSTRACT

Objective: To investigate the effect of ophiopogon−polysaccharide on adiponectin (APN) in gestational diabetic mellitus rat. **Methods:** The model of gestational diabeties mellitus rat were established with streptozotocin by intraperitoneal injection. Model rats were randomly divided into ophiopogon−polysaccharide 125 mg/kg group, 250 mg/kg group, 500 mg/kg group, GDM group and normal pregnancy group (*n*=10). Ophiopogon−polysaccharide was given by intragastric administration for 14 days. The fasting blood glucose levels of rats at the 0, 7, 14 day were detected respectively. The serum insulin level and APN in fat tissue and placenta were detected at the 14 day. **Results:** The fasting blood glucose level, serum insulin level and APN mRNA in fat tissue and placenta were significantly decreased in gestational diabetes rats treated by ophiopogon−polysaccharide compared with model group (*P*<0.05). **Conclusions:** Ophiopogon−polysaccharide could decrease the fasting blood glucose level and serum insulin level, and improve APN mRNA in fat tissue and placenta.

1. Introduction

Pregnancy associated with diabetes including diabetes before pregnancy and gestational diabetes mellitus (GDM) is one of the most common obstetrics complications. GDM refers to abnormal glycometabolism detected at the first time during pregnancy, which considered as one type of diabetes by WHO from 1979 would pose a health risk for mother or babies. Insulin resistance and insulin secretion defection play an important role in the pathogenesis of GDM. Among related genes, adiponectin (APN) gene is of special concern. Clinical practice found that Chinese herb extracts ophiopogon−polysaccharide could lower blood glucose. Here we aimed to investigate the relationship of ophiopogon−polysaccharide on APN in adipose and placenta tissue of gestational diabetic mellitus rat.

2. Materials and methods

2.1. Animals

Wistar rats (male, *n*=25; female, *n*=50) weighted 240−275 g were purchased directly from Animal Center of Guangdong Medical College, and housed in an experimental room under controlled conditions of air change rate (8−12 times/h), temperature (20±2)℃, humidity (55±5)%, noise (below 85 decibels), ammonia concentration (20 ppm), and a 12−hour light/dark cycle with ad libitum access to standard chow and tap water. The bedding was changed and the animals′ were recorded weight every day. After adaptability breeding for 1 week, rats were kept from eating for 12 h then the animal weight was recorded, and fasting plasma glucose was measured with blood obtained via tail prick The animals with fasting plasma glucose≥11.1 mmol/L were excluded. The male rats cohabited with female in estrus for one night, and 50 pregnant rats were obtained. Pregnant rats were assigned randomly into five groups: normal pregnancy group, GDM group, ophiopogon−polysaccharide 125 mg/kg group, 250 mg/kg group, 500 mg/kg group. After pregnancy, diabetes was induced by way of a single subcutaneous

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injection of 2% streptozotocin (STZ) at a dosage of 45 mg/kg. Normal pregnancy animals received a single subcutaneous injection of citrate buffer. Diabetes was defined as a blood glucose concentration ≥11.1 mmol/L, or urine glucose ≥2+.

2.2. Treatments

For diabetic rats receiving ophiopogon–polysaccharide group 125 mg/kg, group 250 mg/kg, group 500 mg/kg, treatment was initiated from day 4 after STZ injection. Normal pregnancy group and GDM group rats received equivalent normal saline.

2.3. Reagent and instrument

STZ (Sigma, USA), citric acid (KeyGEN, Nanjing, China.) ophiopogon–polysaccharide (Rsbio, Shanghai, China), rat–specific ELISA kit for adiponectin, (Puwei, Beijing China.), TRIzol Reagent (invitrogen) primer synthesis (Rsbio, Shanghai, China), RT–PCR kit (TAKATA), I $^{group\ 125}$ Insulin Antibody Radioimmunoassay Kit (Qingdao aohai biology co., LTD. China).

2.4. Method

Blood glucose was measured after 12 h fasting, using glucose oxidase (GOD) method with blood obtained via tail prick. The serum was stored in −20 ℃ refrigerator after 3 000 rpm, 4 ℃ centrifuging 10 min for measurement.

Serum insulin was measured after 12 h fasting, using I group 125 insulin antibody radioimmunoassay kit with blood obtained via tail prick. The serum was stored in −20 ℃ refrigerator after 3 000 rpm, 4 ℃ centrifuging 10 min for measurement.

Adiponectin in serum placental tissue were analyzed using a commercially available ELISA kit, The OD ratio was analyzed using Curve Exert 1.3 software.

The adipose tissue and placental were collected at 14 days after pregnant. The tissue were immediately frozen and stored at −80 ℃ refrigerator measurement. APN–mRNA in adipose tissue and placenta Total RNA from adipose tissue or placenta was isolated with Trizol after grinding in liquid nitrogen. Two microliter RNA was used as a template for Reverse transcription. The total volume of was 20 μ L. cDNA was synthetized at 37 ℃ for 20 min, then 80 ℃ for 10 sec.

Two microliter product was used as a template for PCR amplification The primer was as follows: APN primers: P1–APN: 5′–GGT GCA CCC TTA GGA CCA AGA–3′ P2–APN: 5′–AAA CTT GTG CAG GTT GGA TG–3′, 80 bp; β –actin primers: P1– β –actin:5′–AAC AGT CCG CCT AGA AGC AT–3′,P2– β –actin: 5′–AGC AGA TGT GGA TCA GC AAG–3′, 95 bp. The target gene was semi–quantitative analyzed by using β –actin as internal standard control.

2.5. Statistical analysis

All measurement data are presented as mean±SE. The difference between means was analyzed using *t*–test. All statistical analyses were performed using SPSS 13.0 software. *P* values <0.05 were considered statistically significant. The difference between count data was analyzed by linear correlation analysis, *P* values <0.05 were considered statistically significant.

3. Result

3.1. Change of blood glucose before and after pregnancy

Blood glucose was significantly increased on the 7th and 14th day comparing with that before pregnancy in GDM group and three treatment groups (*P*<0.05). And the level was significantly lower in three treatment groups on 7th day and 14th day comparing with GDM group (P<0.05). And the decrease was more significant as the dosage increasing (Table 1).

3.2. Change of serum insulin before and after pregnancy

Serum insulin was significantly increased after pregnancy comparing with that before pregnancy in GDM group and three treatment groups (*P*<0.05). And the level was significantly lower in three groups comparing with GDM group (*P*<0.05). And the decrease was more significant as the dosage increasing (Table 2).

3.3. Change of adiponectin in 5 groups

The level of adiponectin was significantly decreased in GDM group [(5.31±0.29) μ g/mL] comparing with

Table 1
Change of the blood glucose in 5 groups.

Groups	Before pregnancy	Day 7	Day 14
Normal pregnancy group	5.27±0.74	5.93±1.34	6.21±1.27
GDM group	5.31±0.82	19.79±2.83[a]	24.96±3.51[a]
Ophiopogon–polysaccharide group 125 mg/kg	5.46±0.22	15.87±2.58[ab]	12.21±1.37[ab]
Ophiopogon–polysaccharide group 250 mg/kg	5.09±0.59	13.36±2.07[ab]	11.03±0.96[ab]
Ophiopogon–polysaccharide group 500 mg/kg	5.40±0.83	11.75±3.64[ab]	9.27±3.67[ab]

a: *P*<0.05 comparing with that before pregnancy; b: *P*<0.05 comparing with GDM group.

Table 2

Change of serum insulin in 5 groups (MIU/L).

Groups	Before pregnancy	After pregnancy
Normal pregnancy group	11.36±0.25	11.84±1.34
GDM group	11.28±0.43	40.69±5.57[a]
Ophiopogon–polysaccharide group 125 mg/kg	11.09±0.36	35.87±4.78[a b]
Ophiopogon–polysaccharide group 250 mg/kg	11.47±0.81	30.42±5.07[a b]
Ophiopogon–polysaccharide group 500 mg/kg	11.51±0.73	27.49±6.95[a b]

a: *P*<0.05 comparing with that before pregnancy; b: *P*<0.05 comparing with GDM group.

normal pregnancy group [(13.87±0.94) μ g/mL] (*P*<0.05). After treatment, the level was significantly increased [(7.42±0.85) μ g/mL, (9.98±1.04) μ g/mL, (11.37±1.28) μ g/mL in tree treatment group, respectively] (*P*<0.05).

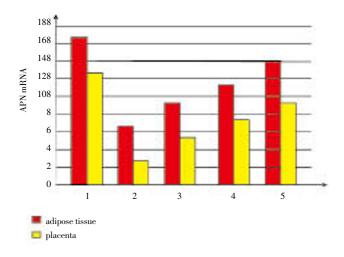*3.4. Change of APN mRNA in adipose tissue and placenta*

Figure 1. Change of APN mRNA in adipose tissue and placenta.
1 normal pregnancy group;
2 GDM group;
3 ophiopogon–polysaccharide 125 mg/kg;
4 ophiopogon–polysaccharide 250 mg/kg;
5 ophiopogon–polysaccharide 500 mg/kg.

The level of APN mRNA was significantly decreased in GDM group comparing with normal pregnancy group in adipose tissue and placenta (*P*<0.05). After treatment, the level was significantly increased in tree treatment group (*P*<0.05) (Figure 1).

4. Discussion

The pathogenetic mechanism of GDM is traditionally attributed to insulin resistance which is due to the effects of prolactin, progestogen, and placental hormones. But recently research shows that other factors such as apiponectin may be concerned. Adiponectin, which is secreted by mature adipocyte, have a significant anti–atherosclerotic effect, and play an important role in glycometabolism and insulin sensitivity[1]. The low level of adiponectin in GDM patient indicates that the change of adiponectin maybe related to the development and progression of GDM[2]. Our study shows

that the adiponectin level in GDM rats was significantly lower than normal rats, meanwhile the adiponectin mRNA in adipose tissue and placenta were also significantly reduced. Adiponectin gene which is located in type 2 diabetes, metabolic syndrome susceptibility loci on human chromosome 3q2 7, is genetic associated. It is composed of the low molecular weight trimer, median molecular weight hexamer, high molecular weight isoform. It contains three exons and two introns about 17 kb[3,4]. Most researchers found that adiponectin gene polymorphism is associated with type 2 diabetes and insulin resistance[5]. Li *et al*[6] found that susceptibility allele to type 2 diabetes in Chinese Han population may be SNP +11377C, SNP +4522T, SNP +45G. Siitonen *et al*[7] found that the increased incidence of type 2 diabetes is related to +3317 allele. Some study found that adiponectin SNP +45 was correlated with the incidence of gestational diabetes and the decrease of adiponectin level[8–11]. Some study also shows that the adiponectin genes +45 T> G and−11377C> G was related with the development of gestational diabetes but have no effect on body mass index in pregnant women with gestational diabetes[12].

Ophiopogon polysaccharide was extracted from Ophiopogon japonicus (Thunb.) Ker–Gawl dried roots. It contains a variety of saponins, such as ophiopogon A, B, B′, C, C′, D, D′, and high level of flavonoid compounds: Ophiopogon flavone A, B, methyl radix ophiopogonis flavonoids A, B, ophiogonanone A, B, methylophiopogonanone, 6–aldehydoisoophiopogonanone and 6–aldehydo–isoophipogonone A, B. It also contains volatile oil, phytosterol, monosaccharide and oligosaccharides. Pharmacological experiments show that it plays an important role in cardiovascular system, and can enhance myocardial contraction and protecte myocardial ischemia. But large doses ophiopogon polysaccharide can inhibit myocardial contractility, reduce coronary blood flow. It also has sedative, hypnotic, anticonvulsant effect, regulates the immune system, and anti–aging[13]. Our study found that the blood glucose in ophiopogon polysaccharide treatment groups is significantly lower than GDM group, while adiponectin in serum, adipose tissue and placenta is significantly higher. It indicate that ophiopogon polysaccharide has a good therapeutic effect on animal models of gestational diabetes. Our study only explored the change of adiponectin in serum, adipose tissue, and placenta after treated with ophiopogon–polysaccharide in animal models. The mechanism of

ophiopogon–polysaccharide is not involved. Our further study will discuss more about the mechanism and the relationship between gestational diabetes and adiponectin gene polymorphism.

Conflict of interest statement

We declare that we have no conflict of interest.

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