Effects of Supplemented Taohe Chengqi Decoction in Treating Insulin Resistance in Rats with Non-Insulin Dependent Diabetes Mellitus

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ABSTRACT
Objective: To investigate the effect of supplemented Taohe Chengqi Decoction (STHCQD) in treating non-insulin dependent diabetes mellitus (NIDDM). Methods: The model of rats with NIDDM was formed with injection of streptozotocin and fed on high calorie diet to study the effects of STHCQD on the release of insulin sensitivity. Results: (1) Fasting serum glucose, serum insulin, intake of food and water were significantly decreased (P < 0.05 – 0.01) in STHCQD-treated diabetic rats as compared with untreated diabetic rats, while the insulin sensitivity was significantly increased (P < 0.05). (2) The liver cell membranes from STHCQD-treated diabetic rats released the quantity of insulin receptor which inhibited adenylate cyclase activity, but this effect was blunted in untreated diabetic rats (P < 0.05). (3) A significantly increased glucose oxidation in adipocyte of STHCQD-treated diabetic rats was found as compared with those of untreated diabetic rats (P < 0.05). Conclusions: STHCQD therapy increased sensitivity and responsiveness of target cells to insulin, i.e. it might decrease insulin resistance at receptor sites and post-receptor sites in rats with NIDDM, but could not reverse the insulin resistance.

KEY WORDS supplemented Taohe Chengqi Decoction, non-insulin dependent diabetes mellitus, insulin resistance, insulin mediator, glucose metabolism, insulin sensitivity

As previous researches have showed, supplemented Taohe Chengqi Decoction (STHCQD, 桃核承气汤) has a good effect in reducing blood glucose and lipid, relieving symptoms in treating patients with non-insulin dependent diabetes mellitus (NIDDM) or NIDDM rats formed by streptozotocin (STZ), and having a tendency in reducing the insulin resistance. Insulin resistance is the significant characteristics of NIDDM which is induced by receptor and post-receptor defect. This experiment has observed the effect on the release of insulin mediator from liver cytomembrane, the glucose oxidation in adipocytes as well as the insulin sensitivity in the experimental NIDDM rats, to investigate the possibility of elevating the target cells to the insulin sensitivity and reactivity namely, in abating the insulin-resistance at receptor site and post-receptor sites.

METHODS

Chemicals and Instruments
STZ, collagenase type II, benzethonium hydroxide, insulin (27.5 u/mg), ATP-disodium salt all are produced by Sigma Chemical Co. cAMP was purchased from Shanghai Biochemical Institute of Chinese Academy of Medical Sciences. D-[U-14 C]-Glucose (248 mCi·mmol⁻¹·L⁻¹) was obtained from Radiological Institute of Chinese Academy of Medical Sciences. I-cAMP test kit was obtained from Isotope Institute of Shanghai University of Traditional Chinese Medicine (TCM). One mol/L phenol reagent was purchased from Apoprotein-Research Department of West China Medical University. Beckman L8-preparative ultracentrifuge, full automatic gel chromatographic apparatus and low-temperature chamber were rented from Molecular Medicine Center of Sun Yat-sen University of Medical Sciences. XSZ-D2 inverted microscope, JEN-1200EX transmission electron microscope, Beckman 5500 automatic counter as well as Beckman Ls 1801 liquid scintillation spectrometer were all obtained from Electron Microscope Laboratory and Nuclear Medicine Department of Guangzhou University of TCM.

Medicine
STHCQD is composed of Radix Astragali, Radix et Rhizoma Rhei, Semen Persicae, Ramulus Cinnamomi, Mirabilitum Depura-
tum, and Radix Rehmanniae\(^{(1)}\). All the herbs were purchased from the Outpatient Pharmacy of our Hospital and verified by Chinese Pharmaceutical Faculty. The decoction was concentrated into the liquid with the concentration equal to 3 g crude drugs per millilitre and kept in refrigerator of \(-4\)°C. Minidiab (glipizide, Farmitalia Crlloerba Ltd Co. Italy) was mixed in normal saline to be a suspension.

**Animals and Grouping**

A total of 100 male SD rats at the age of 10 – 12 weeks with body weight between 140 g and 190 g were provided by Guangdong Medical Animal Center. Seventy-five among them were chosen randomly and injected with STZ and fed on high calorie diet, from which NIDDM model was formed following Liu Yongyu’s method\(^{(4)}\). After 13 weeks’ modeling, 12 of the model rats together with 10 normal rats were sacrificed by decapitation, and the fat-pads of the epididymis were cut down for the study on adipocyte glucose oxidation. Forty-two rats presenting polydipsia, polyphagia, over weight, hyperglycemia, hyperlipemia, hyperinsulinemia and hyposensitivity to insulin were then determined as the experimental NIDDM rats, and divided into Chinese herb group (CHG), western medicine group (WMG) and blank control at random, in addition to normal control with non-modeled rats. Each group were fed with basic forage, CHG added STHCQD with a dose of 25 times as normal adult human dosage (body weight 60 kg), namely 12.5 ml/kg, WHG added Minidiab suspension (5 mg/kg); blank control and normal control both were given normal saline of the equal volume. The administration was applied once every morning and lasted 5 weeks. Detailled record on intake of food and water, body weight and the test on blood sugar, blood lipid and serum insulin taken from orbital vein after 12 hours’ fasting were taken both before and after the treatment. At the end of the experiment, all the rats were killed by decapitation seperately in 4 groups, all the livers were cut down and washed twice in cold normal saline, and then immediately prepared into a liver-cell membrane, meanwhile fat-pads from epididymis and abdomen were collected to isolate fat cells.

**Method**

**Liver-Cell Membrane**

Liver cell membrane was made according to the method from Yan Xiaoqiang\(^{(5)}\). The concentration of membrane protein was measured after Lowry’s method\(^{(6)}\) and 5’-nucleosidase activity by Trouster’s\(^{(7)}\), then a routine slide was made for electron microscopic examination to verify the quality of the extracted liver cell membrane.

**Production and Seperation of Insulin Mediator**

The production and seperation of insulin mediator were achieved by Kang Youhou’s method\(^{(8)}\). The rat’s liver cell membrane was diluted by 10 mmol/L potassium phosphate buffer (pH 7.4) the membrane protein concentrated to 5 g/L, then it was placed in a vial, adding (or not adding) insulin, with final concentration as 2 nmol/L for preservation in 37°C for 5 minutes, then it was immediately put in the ice bath for 10 minutes and followed by centrifugation in 4°C to collect the supernatant fluid, through using column chromatography (sephadex G 25) and washed by 10 mmol/L potassium phosphate buffer (flow rate: 1 ml/10 min). All the components were collected under the monitoring of 230 nm wave, length and the component with highest activity of insulin mediator was chosen to determine the inhibitor action on adenylate cyclase (AC) activity.

**Determination on Insulin Mediator Activity**

Following Kang Youhou’s method, the activity of adenylate cyclase was determined and reflected with unlabelled ATP as substrate whose enzyme acted product-cAMP would be measured by means of extracorporeal RIA. The concentration of cAMP was assayed by RIA reagent kit.

**Determination of Glucose Oxidation in Adipocyte**

The whole process followed Pedersen’s method\(^{(9)}\), and the product of \(^{14}\)CO\(_2\) was absorbed by 0.4 ml 25% benzethonium hydroxide methanol solution.