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Basic Investigation

Effect of houttuynia cordata aetherolea on adiponectin and connective tissue growth factor in a rat model of diabetes mellitus

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Abstract

OBJECTIVE: To determine the effect of Houttuynia cordata Aetherolea on connective tissue growth factor and adiponectin in a rat model of diabetes mellitus (DM).

METHODS: DM was induced in rats using streptozotocin (STZ) and high glucose-lipid animal feed. Animals were then treated with Houttuynia cordata Aetherolea for 8 weeks. Changes in connective tissue growth factor and adiponectin levels in rats were observed.

RESULTS: Connective tissue growth factor and adiponectin levels in rats with DM improved after Houttuynia cordata Aetherolea treatment.

CONCLUSION: Houttuynia cordata Aetherolea had a positive effect on rats with DM by reducing levels of connective tissue growth factor and increasing adiponectin levels.

Key words: Diabetes mellitus; Houttuynia cordata; Connective Tissue Growth factor (CTGF); Adiponectin

INTRODUCTION

Houttuynia cordata is a plant herb belonging to the Saururus chinensis (Lour.) Bail. Houttuynia cordata Thunb. family. According to Chinese medicine, Houttuynia cordata has a cold nature, and is acrid and bitter in flavor. This herbal plant has the ability to relieve superficies by cooling and inducing diuresis to reduce edema. In a clinical setting, it is used to treat lung abscesses, pyemesis, dyspnea associated with cough due to excessive phlegm, micturition disorders, and ulcers. The fresh plant contains volatile oils (0.0049%), and its essential component is Houttuynin, which is also known as decanoylacetaldehyde. The volatile oil of the Houttuynia cordata plant was intragastrically administered to rats with diabetes mellitus (DM) for 8 weeks. We found that Houttuynia cordata reduced urine protein levels and renal lesions in diabetic rats. In order to understand the mechanism involved, we designed a series of experiments to identify the effect of Houttuynia cordata volatile oils on DM rats by monitoring adiponectin and connective tissue growth factor (CTFG) levels. Adiponectin is excreted by adipose tissue and plays an important role in insulin sensitivity and the phlogistic process. In addition, adiponectin has been linked to adipositas cordis, type 2 DM and IR[1], while CTGF has been shown to play an important role in the occurrence and development of kidney hypertrophy. Rosiglitazone functions to decrease the albumin excretion rate, and can markedly improve insulin resistance in diabetic rats[2], while Losartan can reduce urine protein levels during diabetic nephropathy[3], and can also increase insulin sensitivity in diabetic and hypertensive patients[4].

MATERIAL AND METHODS

Animals

Clean male Wistar rats (3 months old), weighing 180-200 g, were provided by the Shanghai Laboratory Animal Center [Shanghai, China, Certification code:

SYXK(沪)2004-0005]. Rats were housed in the SPF Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine under a 12 h light/dark cycle, and had free access to food and water. The center was maintained at a constant temperature and humidity.

Herbal and Commercial Medicines

Houttuynia cordata was obtained from the Shanghai Xuhui Chinese Medicine Decoction Pieces Factory (Shanghai, China). The Houttuynia cordata volatile oil was extracted using the wet distillation method. Losartan (50 mg tablets) was purchased from Merck & Co., Inc. (Hangzhou, China), Rosiglitazone (4 mg tablets) was purchased from GlaxoSmithKline (Tianjin, China), and streptozotocin (STZ) was from Sigma-Aldrich Co. (St. Louis, MO, USA).

Animal Groupings

Sixty male Wistar rats, after 1 week of adaptive feeding, were divided into 3 groups. The mockup group (n=51) was fed a high-carbohydrate and high-fat diet[5] (20% (w/v) sucrose, 10% (w/v) axungia porci, 2.5% (w/v) cholesterol, and 67.5% (w/v) common diet) every day, and had free access to drinking water. The control group (n=9) was fed the common diet. After 30 days, the mockup group was food restricted for 12 h. STZ was dissolved in 0.1 mol/L citric acid buffer (pH 4.0) to a concentration of 0.1% (w/v). The STZ solution was then intraperitoneally injected into rats at a dose of 30 mg/kg. After 72 h, fasting blood sugar and urine glucose were detected using the one touch II glucometer and glucose reagent strips (Lifescan Inc., Milpitas, CA, USA), and the Hitachi type 7170 automatic biochemistry analyzer (Hitachi Ltd., Tokyo, Japan), respectively. Successful induction of DM in 50 rats was achieved when blood sugar levels were > 16.7 mmol/L and urine protein output increased by 50% when compared with data before mockup. Equivalent amounts of citric acid buffer were intraperitoneally injected into control animals. Rats in the mockup group were randomly divided into 5 sub-groups: 1) Houttuynia cordata water solution group (5400 mg/kg/day Houttuynia cordata in 2 mL water), 2) Houttuynia cordata volatile oil group (5400 mg/kg/day 2 mL Houttuynia cordata volatile oil suspension), 3) Losartan group (30 mg/kg/day Losartan, dissolved in 2 mL water), 4) Rosiglitazone group (30 mg/kg/day Rosiglitazone dissolved in 2 mL water), and 5) model group (given equivalent amount of water).

Detection Index

Changes in drinking consumption, food consumption, and body weight of all animals were monitored at the end of the fourth and eighth week. At the end of the eighth week, rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg). Blood samples were collected from rats to measure fasting plasma glucose (FPG), fasting serum insulin (FINS), blood serum adiponectin, and CTGF levels and the insulin sensitivity index. The kidneys of rats were resected to measure tissue adiponectin levels using the enzyme linked immunosorbent assay (ELISA). The insulin sensitivity index (ISI) was calculated as follows.

ISI=-Ln1/FINS*FPG[6]

Statistical Analysis

All data were analyzed using SPSS 10.0 (IBM, Armonk, NY, USA), and are expressed as the mean ± s.e.m. Statistical analyses were performed using one-way analysis of variance. When heterogeneity of variance appeared, the Dunnet T3 multiple comparison test was used. A value of $P<0.05$ was considered statistically significant.

RESULTS

Rats in the control group had good mental status and remained active (they were alert and their pelt was glossy). After 1 month on a high-carbohydrate and high-fat diet, the body weight and food intake of rats in the mockup group increased. After injection with STZ for 4 weeks, food intake remained high, but body weight only increased slightly when compared with the control group. The weight of rats in the model group decreased after injection with STZ, but rats suffered from polydipsia and polyuria, were mentally dispirited, were unresponsive, their pelt was no longer glossy, and they became slow. Animals in all the therapeutic sub-groups of the mockup group showed better mental condition and agility, but were less responsive when compared to the control group.

FPG levels in the model group, Houttuynia cordata water solution group and the Losartan group were significantly higher than the control group ($P<0.05$; Table 1). There were no significant differences between any of the sub-groups of the mockup group. All therapeutic sub-groups had significantly lower blood glucose levels when compared with the model group ($P<0.05$; Table 1). In the model, Houttuynia cordata water solution and Losartan groups, FINS levels were significantly increased when compared to the control group ($P<0.05$; Table 1). At the same time, high blood glucose levels existed in these diabetic rats, indicating that there was insulin resistance.

FINS levels in the Houttuynia cordata volatile oil group and Rosiglitazone group were significantly lower ($P<0.05$; Table 1) than the model group. Moreover, FINS levels in the Houttuynia cordata water solution and Losartan groups were significantly higher when compared with the Houttuynia cordata volatile oil and Rosiglitazone groups ($P<0.05$; Table 1).

The ISI for the Houttuynia cordata volatile oil group and Rosiglitazone (insulin sensitizer) group was significantly higher when compared with the model and Losartan groups ($P<0.05$; Table 1).

Table 1 Influence of Houttuynia cordata Aetherolea on FPG, FINS, and the ISI in diabetic rats ($\bar{x}$ ±s)

Group	n	dosage	FPG/mmol·L^{-1}	FINS/μ·mL^{-1}	ISI
Control	9	-	6.01±1.13	23.01±4.75	-4.34±0.49
Model	10	-	11.59±4.21$^{\#}$	37.52±8.85$^{\#}$	-6.99±0.50$^{\#\triangle+}$
Houttuynia cordata water solution	10	5400 mg·kg^{-1}·d^{-1}	9.02±2.24$^{\#*}$	34.16±12.89$^{\#\triangle+}$	-5.64±0.53$^{\#}$
Houttuynia cordata volatile oil	10	5400 mg·kg^{-1}·d^{-1}	7.75±1.96*	27.52±5.47*	-5.31±0.38$^{\#}$
Losartan	10	30 mg·kg^{-1}·d^{-1}	8.21±2.20$^{\#*}$	37.21±13.17$^{\#\triangle+}$	-5.99±0.32$^{\#\triangle+}$
Rosiglitazone	10	3 mg·kg^{-1}·d^{-1}	7.53±1.75*	25.13±4.34*	-5.12±0.18$^{\#}$

Note: $^{\#}P$<0.05 vs. control group; $^{*}P$<0.05 vs. model group; $^{\triangle}P$<0.05 vs. Houttuynia cordata volatile oil group; ^{+}P<0.05 vs. Rosiglitazone group.

CTGF levels in the model group and the Western medicine groups were significantly higher than the control group (P<0.05; Table 2). However, levels in both the Houttuynia cordata water solution group and Houttuynia cordata volatile oil group were significantly lower than the Western medicine and model groups (P< 0.05; Table 2).

Serum and tissue adiponectin levels in the model and all therapeutic groups were lower than the control group. However, adiponectin levels in the Houttuynia cordata water solution group and Houttuynia cordata volatile oil group were significantly higher than the model group (P<0.05; Table 2). Adiponectin levels in the Rosiglitazone groups was significantly lower than in the Houttuynia cordata volatile oil group (P<0.05; Table 2).

Table 2 Serum/tissue levels of adiponectin and CTGF in diabetic rats ($\bar{x}$ ±s)

Group	n	Tissue adiponectin (μg/mL)	Serum adiponectin (μg/mL)	CTGF (pg/mg)
Control	9	14.32±3.35	5.07±1.38	6.10±0.69
Model	10	10.13±2.86$^{\#}$	2.83±0.85$^{\#}$	9.14±0.3$^{1\#}$
Houttuynia cordata water solution	10	13.18±4.23*	4.29±1.57*	6.39±0.52*
Houttuynia cordata volatile oil	10	13.68±3.04*	4.73±0.74*	6.70±0.64*
Losartan	10	11.81±2.34$^{\#\triangle}$	4.27±1.45*	8.03±1.12$^{\#*\triangle}$
Rosiglitazone	10	11.45±1.49$^{\#\triangle}$	3.87±0.76$^{\triangle}$	7.70±2.06$^{\#*\triangle}$

Note: $^{\#}P$<0.05 vs. control group. $^{*}P$<0.05 vs model group. $^{\triangle}P$<0.05 vs. Houttuynia cordata volatile oil group.

DISCUSSION

Houttuynia cordata volatile oil was extracted by distillation from the Houttuynia cordata plant. The essential component of this herb is Houttuynin, which has bacteriocidal, antiblastic, antivirotic, anti-inflammatory, urinative, and antilipemic effects, and has an anti-blood platelet congregating function. Some studies have shown that Houttuynia cordata can inhibit hypertrophy of the kidney glomerulus, decrease urea β_2-microglbulin levels, the urea albumin excretion rate and creatinine clearance rate within 24 h[7]. Rosiglitazone functions to decrease the albumin excretion rate[8], and can markedly improve insulin resistance in diabetic rats[9-10], while Losartan can reduce urine protein levels during diabetic nephropathy[3], and can also increase insulin sensitivity in diabetic and hypertensive patients [4]. In this study, we identified how Houttuynia cordata may improve diabetic nephropathy via the following mechanisms:

Improvement of insulin resistant (IR)

The development of DM is very complex. Currently, DM is believed to be related to IR and/or the lack of beta cell insulin secretion. The insulin required to stimulate skeletal muscle intake of glucose is more than the quantity of insulin needed to inhibit the production and output of hepatic glycogen[11]. Therefore, postprandial blood glucose increases, and consequently fasting blood glucose increases. When IR becomes serious, beta cell failure, due to long term overcompensation, and disruption to glycometabolic control is gradually exacerbated. When blood glucose levels increase and continue to persist, DM can occur[12].

IR can be regarded as the efficiency of insulin to mediate glucose homeostasis. The glucose metabolic rate measured by the high glucose clamp technique is a common testing standard[13]. However, a more convenient and economic index is needed for epidemiological studies.

It is well known that when hyperinsulinism and high bold glucose occur together, IR occurs. The insulin sensitive index, which is referred to as the cross product of FINS and FPG, for example, the HOMA-IR founded by Mathews[14], 1/FPG*FINS founded by Liguangwei in 1993 and QUICKI[1/(logFPG*logFINS)] founded by Katz in 2000[15], largely represents the insulin sensitivity of an organism. Therefore, insulin sensitivity can be used as an index in epidemiology to observe the association of IR and other factors. The results from our experiment revealed that Houttuynia cordata treated groups had improved insulin sensitivity, and had similar effects to Rosiglitazone, an insulin sensitizer

that has been shown to have curative effects[2].

Changes in Adiponectin Levels

Adiponectin, which is excreted by adipose tissue, plays an important role in insulin sensitivity and the phlogistic process, and has been linked to adipositas cordis, type 2 DM and IR[1]. Some studies have shown that patients with adipositas cordis, type 2 DM and cardiovascular diseases have IR and hyperinsulinism simultaneously[16]. In addition, the concentration of adiponectin in the plasma of these patients was observed to decrease[17]. During the development of type 2 DM, the reduction of adiponectin concentrations in plasma is equal to the reduction in IR[18]. Adiponectin can be excreted from mature adipose tissue, and adipocytokines, which have an anti-inflammatory function, increase insulin sensitivity and have a cardioprotective effect. In addition, adiponectin can inhibit apoptosis of beta cells induced by inflammatory factors[19]. The reduction of adiponectin is a key link to type 2 DM[20]. When patients began to have IR, their serum adiponectin levels significantly decreased[21-22].

Our results showed that adiponectin levels in the serum and tissue of animals in the model group and all therapeutic groups were lower than the control group, which is similar to a previous report[23]. Adiponectin levels in both herbal medicine groups, particularly the Houttuynia cordata volatile oil group, and both Western medicine groups, were markedly higher than the model group. These results indicate that Houttuynia cordata can improve adiponectin levels in rats with DM.

CTGF Expression

CTGF is highly abundant in the tissues and organs of humans, especially in the kidney, which has the highest CTGF concentration. CTGF has many biological functions, including promoting cell multiplication, adjusting extracellular mediators, mediating cell adhesion, promoting cell phenotype transformation and chemotaxis effects. During early stages of DM, kidney hypertrophy is a characteristic pathological change[24]. CTGF plays a very important role in the occurrence and development of hypertrophy. An in vitro study has shown that CTGF not only induces the synthesis of the extracellular matrix (ECM) in interstitial cells, but also mediates the creation of enzyme inhibitors stimulated by transforming growth factor beta, which leads to an increase in the synthesis of the ECM and a decrease in degradation[25]. Recent clinical research confirmed that urea and blood CTGF concentrations and the degree of severity of diabetic nephropathy (urinary albumin excretion rate) are directly proportional to glomerular filtration rate[26]. CTGF acts as a fibrosis factor in the progressive fibrosis of the kidney in diabetic nephropathy by promoting cell mitosis, adjusting the synthesis of the ECM, and adjusting the restriction point of the cell cycle [27].

In vitro experiments have also demonstrated cellular adhesion in rats subjected to TGF-β or high glucose conditions, where TGF-β mRNA and albumin levels increased. Furthermore, the addition of recombinant CTGF to cell culture medium can induce the synthesis of the ECM, indicating that high glucose can promote the process of kidney fibrosis in diabetic nephropathy by promoting the production of CTGF[28]. In this study, we showed that Houttuynia cordata volatile oil can reduce CTGF levels, which may be one of the mechanisms of relieving kidney injury in diabetic rats.

Currently, there is great interest in the effect of Chinese medicine on diabetic nephropathy. However, few studies have investigated the effect of Houttuynia cordata. Our study has shown that blood glucose, insulin, adiponectin and CTGF levels in diabetic rats, induced by the combination of a high-carbohydrate and high-fat diet, and STZ injection, were affected by Houttuynia cordata volatile oil, which may function by reducing IR, adiponectin and CTGF levels. Thus, Houttuynia cordata volatile oil may have potential therapeutic effects for relieving kidney injury in diabetic rats.

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