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The effect of Liuwei Dihuang decoction on PI3K/Akt signaling pathway in liver of type 2 diabetes mellitus (T2DM) rats with insulin resistance

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Chemical compounds studied in this article:
Morroniside($C_{17}H_{26}O_{11}$,402.38)
Sweroside($C_{16}H_{22}O_9$,358.34)
Paeonoside($C_{23}H_{28}O_{11}$, 480.47)
Loganin(:$C_{17}H_{26}O_{10}$,390.38)

ABSTRACT

Ethnopharmacological relevance: Liuwei Dihaung decoction (LWDHT) is a well-known classic traditional Chinese medicine formula, consists of six herbs including Rehmannia glutinosa Libosch.(family: Scrophulariaceae), Cornus officinalis Sieb.(family: Cornaceae), Dioscorea opposite Thunb.(family: Dioscoreaceae), Alisma orientale(G. Samuelsson) Juz (family: Alismataceae), Poria cocos (Schw.) Wolf (family: Polyporaceae) and Paeonia suffruticosa Andrews (family: Paeoniaceae). It has been used in the treatment of many types of diseases with signs of deficiency of Yin in the kidneys in China clinically. This study is aimed at investigating the effect of Liuwei dihuang decoction on PI3K/Akt signaling pathway in liver of T2DM rats with insulin resistance.

Materials and methods: T2DM model was induced in male Sprague-Dawley (SD) rats by high sugar and high fat diets combined with small dose of streptozocin (STZ) injection. The successful T2DM rats were randomly allocated three group–vehicle group, positive control group and Liuwei Dihuang decoction group. After 12-weeks treatment with distilled water, rosiglitazone and LWDHT by intragastric administration respectively, the rats were put to death in batches. The variance of fasting blood glucose (FBG) and fasting insulin (FINS) in serum were determined, the pathological changes of each rats' liver were observed by hematoxylin-eosin (HE) staining, the expression of insulin receptor substrate 2(IRS2), phosphatidylinositol 3-kinase (PI3K) and protein kinas B (Akt) involving the canonical PI3K/Akt signaling pathway were detected by Real-time fluorescent quantitative PCR (RT-PCR), and the expression level of IRS2, PI3K, Akt protein and phosphorylated IRS2, PI3K, Akt protein were evaluated by Western Blot. All the data were analyzed by SPSS 17.0.

Results: Four weeks of treatment with LWDHT could significantly decrease the level of FBG and FINS in serum, improve the cellular morphology of liver, kidney, pancreas tissue, and the expression of IRS2, PI3K, Akt mRNA and phosphorylated IRS2, PI3K, Akt protein involved in the canonical PI3K/Akt signaling pathway of T2DM rats in liver were significantly up-regulated, while the total IRS2, PI3K, and Akt protein had no obvious changes.

Conclusions: The results suggest that Liuwei Dihuang decoction could intervene insulin resistance of T2DM, in part, through regulation of canonical PI3K/Akt signaling pathway of T2DM rats in liver.

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1. Introduction

Diabetes mellitus (DM), characterized by chronic hyperglycemia, is a kind of metabolic disorders caused by various etiology, whereas type 2 diabetes mellitus (T2DM) is mainly due to the decrease of insulin secretion and the defects with insulin resistance (IR) of the target tissues (liver, skeletal muscle and adipose tissue, which accounted for 90–95% (Whiting et al., 2011). IR is a physiological condition in which cells fail to respond to the

Abbreviations: IRS2, insulin receptor substrate 2; PI3K, phosphatidylinositol 3-kinase; Akt, protein kinase B; T2DM, type 2 diabetes mellitus

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normal actions of the hormone insulin. IR often appears before the secretion of insulin. When insulin secretion of β cells can compensate IR, it will not lead to hyperglycemia and IR itself is not enough to induce type 2 diabetes, but when the body can not compensate for the increased blood glucose caused by IR, it will lead to the occurrence of type 2 diabetes. In recent years, diabetes is prevalent widespread, and it is ranked third of chronic diseases after tumor, cardiovascular disease. Currently, hypoglycemic drugs have already existed as insulin, insulin secreting drugs, insulin sensitizing agents, etc. However, there are a series of adverse reactions such as hypoglycemia, gastrointestinal discomfort. Now it still lacks of effective means to control the occurrence and progress of diabetes as well as its complications.

Preventive treatment of disease with traditional Chinese medicine has a long history in China and many kinds of components in Chinese herbal medicine with pharmacological activities act synergistically on patients, which represents the traditional model of combined treatment (Liu et al., 2013; Pari et al., 2014). In recent years, the study of Liuwei Dihaung decoction has gone into deeper, which is earliest recorded in the book of "Xiaoer YaoZheng ZhiJue". The pharmacological test showed that this formula can improve insulin resistance (Perry et al., 2012), resist to apoptosis and have antioxidation (Perry et al., 2014).

Insulin mainly regulates blood glucose by PI3K/Akt (phosphoinositide-3-kinase/protein kinase B) signaling pathway (Wang et al., 2013). When animals or human beings eat something, the insulin will be produced by beta cells pass into the bloodstream and affects insulin receptor on the surface of the liver cell membrane, which activates the phosphorylated tyrosine located in the beta receptor subunit (White, 2002). Then the activated insulin receptor phosphorylates tyrosine site of insulin receptor substrate-1/2 (IRS-1/2) (the insulin metabolism effects in liver is mainly due to IRS2), which activates PI3K. And the activated PI3K can catalyze 4, 5–2 phosphatidyl inositol phosphate (PIP2) and generate PIP3, which may act the second messenger to activate Akt. Finally, the activated Akt has effects on the biological metabolism by regulating downstream molecules.

Recent studies have found that the IRS2 knockout rats manifest as the symptoms of severe diabetes (Domimic et al., 1998); where PI3K is closely associated with the IR (Morino et al., 2008). The studies (Kemue et al., 1997) have shown that the amount of p85 subunit of PI3K is lower nearly half than normal group in T2DM rats' liver cells. The phosphorylation level of stimulated PI3K-Akt is a major index that reflects the activity of Akt pathway, where Akt Ser473 is the major Akt phosphorylation form after PI3K is activated. Under insulin resistance of diabetes, phosphorylation level of Akt Ser473 decreases (Bozulic and Hemmings, 2009), and insulin signal transduction subsides. Thus, any abnormity appeared along PI3K/Akt signaling pathway will affect the transduction of insulin signal, prompting the development of IR as well as T2DM.

In this study, the T2DM model was established by feeding rats with high sugar and high fat diet in combination with small dose of streptozocin (STZ) injection, then explored the effect of Liuwei Dihuang decoction on PI3K/Akt signaling pathway of T2DM rats with insulin resistance and potential molecular mechanisms underlying the action of the insulin resistance in T2DM, which is hopeful to find new targets for clinical treatment of T2DM.

2. Materials and methods

2.1. Preparation of LWDHT

The preparation of LWDH was the same as the methods showed in our previous study (Dai et al., 2012). In brief, Rehmannia glutinosa Libosch. (family: Scrophulariaceae), Cornus officinalis Sieb. (family: Cornaceae), Dioscorea opposite Thunb. (family: Dioscoreaceae), Alisma orientale(G. Samuelsson) Juz (family: Alismataceae), Poria cocos (Schw). Wolf (family: Polyporaceae) and Paeonia suffruticosa Andrews (family: Paeoniaceae) were mixed as a proportion of 8:4:4:3:3:3, and the total dry weight was 1 kg. The mixture was decocted in 6 L distilled water for 30 min twice. The water extracts were concentrated to 1.915 g/ml for use. All the plants were provide by Pharmacy of The First Affiliated Hospital.

2.2. HPLC analysis

An Agilent-1100 HPLC system (Agilent Technologies, MA, USA) equipped with quaternary pump and UV detection system. A C18 Hypersile ODS2 column (250 mm × 4.6 mm, 5 μm, Dalian Elite Analytical Instruments Co., LTD, China) were used, the mobile phase consisted of methanol-water(27:73), the detective wavelength was 236 nm, the flow rate was 1.0 ml/min, the column temperature was 25 °C.

2.2.1. Standard solutions

Each accurately weighed standards were dissolved in methanol respectively, all the standard solutions were stored in the refrigerator at 4 °C before analysis.

2.2.2. Sample solutions

Take part of the LWDHT treated with 70% ethanol as alcohol sink, and the ethanol-soluble portion was decompression drying to concentrate up to dryness. One gram pulverized samples were accurately weighed and ultrasonically extracted with 10 ml 40% methanol for 60 min in a conical flask, and then cooled to room temperature. The supernatant filtrated through a syringe filter (0.45 μm) were subjected to HPLC before analysis.

2.3. In vivo study design

2.3.1. Animal model and drug administration

Male SPF SD rats weighed about 200 ± 20 g were used in this study (n=70). All the animals were purchased from human SJA co., LTD., and kept in the laboratory center of the first affiliated hospital of Hunan University of Chinese Medicine(Agreement Number: SCXK-xiang-2011–0003). After feed for 1 week, they were randomly divided them into blank control group (n=6) and vehicle group (n=64), giving normal chow diet (provided by the animal department of Hunan University of Chinese Medicine) and high sugar and high fat diet, respectively (Perry et al., 2014). After 4 weeks feeding, all animals were fasted for 12 h. Rats in vehicle group were intraperitoneal injected with 1% STZ(45 mg/kg) and blank control group were intraperitoneal injected with isopyknic citrate disodium hydrogen phosphate buffer. Seventy two hours after injected with STZ, the bloods of rats were collected for detecting FBG and FINS, rats with the FBG ≥ 16.7 mmol/l (Haimei et al., 2011) with reduced ISI will be considered as successful T2DM model. Then, among the successful T2DM rats, 42 rats were randomly divided into three groups as follows: vehicle group (model blank control group), positive group and LWDHT group (n=14 per group). The rats in LWDHT group were given LWDHT (6.75 g/kg) by intragastric administration once per day, and the positive group was given rosiglitazone (0.81 mg/kg, CHONGQING tai ji INDUSTRY co., ltd., chongqing, China), while same amount of distilled water was used in the blank control group and the model blank control group was dealt with the same procedure. After 30 days of treatment, all rats were fasted for 10 h to detect the FBG, and sacrificed to death. The liver of rats were collected, weighed and put in liquid nitrogen for a quick freeze, and then stored in −80°C.

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