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Research Paper

Metabolomic and lipidomic study of the protective effect of Chaihuang-Yishen formula on rats with diabetic nephropathy

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ABSTRACT

Ethnopharmacological relevance: Chaihuang-Yishen formula (CHYS) is a Chinese herbal formula that has been shown clinically to effectively treat chronic kidney disease including diabetic nephropathy (DN), also known as diabetic kidney disease. Our previous animal studies showed that numerous intrarenal metabolites were associated with the development of DN. In the present work, an integrated metabolomic and lipidomic analysis was used to further examine whether CHYS could attenuate the development of DN by regulating the disordered metabolic pathways.

Method: Progressive diabetic kidney disease was induced in Wistar rats by uninephrectomy and a single intraperitoneal injection of streptozocin. Over 20 weeks, one group of animals was treated with CHYS and another group went untreated. Effects of CHYS on metabolomic and lipidomic changes in the renal cortex of diabetic rats were studied using gas chromatography/time-of-flight mass spectrometry, ultra-performance liquid chromatography/time-of-flight mass spectrometry, and tandem MS-based metabolomic and lipidomic. The well-established drug fosinopril was used as positive control throughout the experiment.

Results: Like fosinopril, treatment with CHYS produced a renoprotective effect against DN. Metabolomic and lipidomic analyses showed that the therapeutic effect of CHYS on DN was significantly associated with inhibition of the elevated organic toxins including several uremic toxins and glucuronides, and normalization of diminished phospholipids, especially sphingomyelins.

Conclusion: Improved abnormal metabolic and lipidomic disorders, such as accumulation of uremic toxins and glucuronides and phospholipids, may be mechanisms by which treatment of CHYS inhibits DN. Results from this study provide new evidence for the pharmacologic characteristics of CHYS on DN.

1. Introduction

Diabetic nephropathy (DN), also known as diabetic kidney disease, is a common microvascular complication of diabetes mellitus and one of the major causes of end-stage renal disease worldwide (Sun et al., 2013). DN is characterized by a series of renal structure abnormalities, including basement membrane thickening, mesangial expansion, glomerulosclerosis, and tubulointerstitial fibrosis (Abe et al., 2011). The pathogenesis of DN is complex with systemic metabolic abnormalities (Cohen et al., 2008). Application of metabolomics in the field of metabolic diseases, including diabetes mellitus (DM), has aroused worldwide attention in the past few years (Bao et al., 2009; Li et al., 2007a; Wikoff et al., 2007; Xia et al., 2009; Zhang et al., 2009; Zhao et al., 2012b). As well, lipidomics has also been regarded as a major contributor to our understanding of biological processes in metabolic diseases, such as DM (Haus et al., 2009) and DN (Han et al., 2011). In our previous studies, metabolomic (Zhao et al., 2012b) and lipidomic (Zhao et al., 2012a) analyses were used to explore the effect of local metabolic disorders on the development of DN in streptozocin (STZ)-induced diabetic rats. A number of the identified abnormal

Abbreviations: DN, diabetic nephropathy; CHYS, Chaihuang-Yishen formula; STZ, streptozotocin; GC–TOF MS, gas chromatography/time-of-flight mass spectrometry; UPLC–TOF MS, ultraperformance liquid chromatography/time-of-flight mass spectrometry; PLS-DA, partial least square-discriminant analysis; TCM, traditional Chinese medicine.

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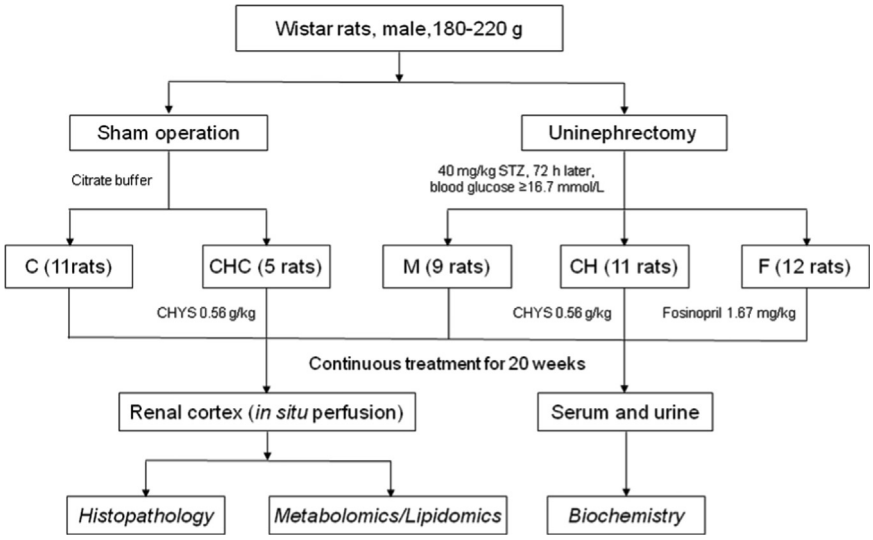

Fig. 1. Schematic of animal experiment design. C: control group; M: model group; CH: CHYS group; F: fosinopril group; CHC: CHYS treated nondiabetic group.

metabolites, such as some uremic toxins and sphingomyelins, exhibited remarkable association with the level of 24-h urinary protein and the degree of tubulo-interstitial injury among diabetic and nondiabetic rats.

Traditional Chinese medicine (TCM) has been practiced for thousands of years in China with increasing awareness of its application in the prevention and treatment of complex multifactor diseases, such as DN (Xie et al., 2011; Xu et al., 2012). In Chinese medicine the symptomatology of chronic kidney disease corresponds with a TCM syndrome known as deficiency of *qi* (vital energy) and blood, complicated by the pathogenic factors of blood stasis, phlegm-dampness, and toxic heat, ultimately resulting in an imbalance of *yin* and *yang* in the body. To treat this syndrome, the TCM therapeutic principle is to replenish *qi* and nourish blood, clear heat and eliminate dampness, and re-balance *yin* and *yang*.

Chaihuang-Yishen formula (CHYS, 柴黄益肾方), also known as Qilong-Lishui formula (芪龙利水方), was originated from eminent Chinese physician Zhensheng Shi's clinical experience in treating CKD (Li, 2005) and ancient classical formula Sairei-to, first appeared in the medical book of "Shi-yi-de-xiao-fang (世医得效方)" at AD.1337. On the basis, we developed CHYS and conducted a series of experimental research related to CKD (Wang et al., 2006). The TCM properties of CHYS are supplementing *qi*, activating blood circulation, and promoting diuresis. Thus, this formula has been used in China in the treatment of chronic kidney disease with marked effect in decreasing proteinuria (Li et al., 2007b). Our recent studies found that CHYS could also ameliorate renal injury in diabetic rats through reduction of inflammatory cytokines (Zhang et al., 2014) and fibrosis (Zhao et al., 2014). To further examine whether CHYS could attenuate the development of DN by regulating the disordered metabolic pathways, an integrated metabolomic and lipidomic experiment was designed on the basis of our previous work. The goal was to identify metabolic signatures associated with CHYS intervention in the renal cortex of rats with DN induced by STZ.

2. Materials and methods

2.1. Preparation of CHYS

CHYS consists of seven crude herbs: *Bupleurum chinense* DC (*chai hu*; bupleurum); *Astragalus membranaceus* (*Fisch.*) Bge. Var. *mongholicus* (Bge.) Hsiao. (*huang qi*; astragalus); *Angelica sinensis* (Oliv.) Diels (*dang gui*; tangkuei); *Dioscorea nipponica* Makin (*chuan long shu yu*; Japanese diascorea); *Polyporus umbellatus* (Pers.) Fries (*zhu ling*); *Pyrrosia petiolosa* (Christ) Ching (*shi wei*; pyrrosia); and *Hirudo nipponica* Whitman (*shui zhi*; leech). The crude herbs were purchased from Beijing Tong Ren Tang Group Co. Ltd. (Beijing, China) and identified by the botanist Professor Zongwan Xie of the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. Furthermore, the plant names have been checked with the website www.plantlist.org.

CHYS was prepared as previously described (Li et al., 2007b; Zhao et al., 2014). The chemical fingerprint of CHYS was previously established by high performance liquid chromatography (HPLC), and the main components of CHYS identified mainly as protocatechuic acid, chlorogenic acid, calycosin 7-O-β-D-glucoside, formononetin, and dioscin (Zhao et al., 2014). These were used for quality control since their contents are relatively constant.

2.2. Experimental animals

The flowchart of animal experiments is shown in Fig. 1. All experiments were conducted with 6-week-old male Wistar rats weighing 180–220 g purchased from Beijing HFK Bio-Technology Co. Ltd. (Beijing, China; Certificate no. SCXK 2002-0010). Animals were housed in a 12-h light/dark cycle environment with free access to food and tap water under a temperature of 20–25 °C and humidity of 65–69%. Thirty-five rats were anaesthetized by intraperitoneal injection with chloral hydrate (330 mg/kg body weight), and the right kidney was removed through a flank incision to accelerate development of DN. We also prepared sham-operated rats, which underwent a similar flank incision followed by kidney exteriorization only. Animals were randomly divided into sham control (Group C, $n=11$) and sham control treated with CHYS at a dosage of 0.56 g/kg-body weight/day (Group CHC, $n=5$). Following the operation, uninephrectomized rats were injected intraperitoneally with streptozotocin (STZ; 40 mg/kg, Sigma, St. Louis, MO, USA) dissolved in 0.1 mol/L sodium citrate buffer (pH 4.0) to induce diabetes. Seventy-two hours after the injection, uninephrectomized STZ-injected rats with blood glucose level ≥ 16.7 mmol/L were considered diabetic. Diabetic rats were randomly divided into three groups. One group was assigned to CHYS at a dosage of 0.56 g/kg-body weight/day (Group CH, $n=11$). Another group was given fosinopril at a dosage of 1.67 mg/kg-body weight/day (Group F, $n=12$). The final group was given an equal volume of distilled water (Group M, $n=9$). All drugs