Journal of Ethnopharmacology 192 (2016) 201–209

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Tongqiaohuoxue decoction ameliorates obesity-induced inflammation and the prothrombotic state by regulating adiponectin and plasminogen activator inhibitor-1

Soon-Hee Kim [a], Hee-Sook Park [a], Moon Ju Hong [a,b], Ji Young Yoo [a], Hoyoung Lee [c], Ju Ah Lee [c], Jinyoung Hur [a], Dae Young Kwon [a,b], Myung-Sunny Kim [a,b,*]

[a] Division of Metabolism and Nutrition, Korea Food Research Institute, Gyeonggi-do 13539, Republic of Korea
[b] Department of Food Biotechnology, University of Science and Technology, Gyeonggi-do 13539, Republic of Korea
[c] KM Fundamental Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, Republic of Korea

ARTICLE INFO

Article history:
Received 3 December 2015
Received in revised form
29 June 2016
Accepted 7 July 2016
Available online 9 July 2016

Keywords:
Tongqiaohuoxue decoction
Inflammation
Obesity
Prothrombotic state
Adiponectin

ABSTRACT

Ethnopharmacological relevance: Tongqiaohuoxue decoction (THD), a water extract of a mixture of eight species of medicinal herbs, has been used for the treatment of blood stasis and hypercoagulation in traditional East Asian medicine since 18th century.
Aim of the study: To investigate the *in vivo* efficacy of THD using high-fat diet (HFD)-induced obese mice with chronic inflammation and a prothrombotic state as an early vascular model.
Materials and methods: THD was prepared by hot water extraction and freeze-drying. Male C57BL/6 mice were divided into three groups. Group 1 (NC) mice were fed normal chow. Mice in group 2 (HFD) and 3 (HFD+THD) were fed with HFD for 12 weeks. In addition, Group 3 mice were administered with 100 mg/kg body weight THD for 4 weeks after onset of obesity by HFD for 8 weeks. Glucose tolerance tests and histological tissue examinations were performed. The levels of adipokines, inflammatory markers, and prothrombotic markers were assessed.
Results: The oral administration of THD for 4 weeks had no effect on the liver, adipose tissue, or total body weight when the HFD and HFD+THD groups were compared. Nevertheless, mice treated in THD interestingly showed a significant increase in adiponectin in blood and adipose tissue. To verify the effect of THD on adiponectin, 3T3-L1 adipocytes were treated with THD; it stimulated adiponectin production in a dose-dependent manner. In the HFD+THD group, pro-inflammatory cytokines were significantly down-regulated in the blood, adipose tissue, and liver. Insulin resistance was also notably improved by THD. Simultaneously, THD significantly reduced plasminogen activator inhibitor-1 (PAI-1) levels in serum, adipose tissue, and liver. Fibrin deposition and tPA activity, downstream targets of PAI-1, were also notably reduced in the HFD+THD group compared to the HFD group.
Conclusions: THD improved obesity-induced inflammation and insulin resistance by increasing adiponectin production. Additionally, THD administration exerted an anti-thrombotic effect through the regulation of PAI-1 and fibrinolysis. This study demonstrates the efficacy of a traditional East Asian medicine by providing scientific evidence and suggesting a possible mechanism of action.

1. Introduction

Tongqiaohuoxue decoction (THD, Tonggyuhwalhyeol-tang in Korea), a classical prescription in traditional East Asian medicine, has been used for the prevention and treatment of cerebrovascular disease (CVD), especially for cases in which the symptoms are felt around the head and neck (HR, 1996). Since THD was established by Wang Qingren (1768–1831), a physician in the late Qing Dynasty, as a hot extract of a mixture of 8 species of medicinal herbs (Wang et al., 2012), it has been prescribed in East Asian countries,

Abbreviations: THD, Tongqiaohuoxue decoction; HFD, high-fat diet; HFD+THD, THD-treated mice on a HFD; PAI-1, plasminogen activator inhibitor-1; TNF, tumor necrosis factor; MCP-1, monocyte chemoattractant protein-1; IL, interleukin; TG, triglyceride; tPA, tissue-type plasminogen activator; GTT, glucose tolerance test; CLS, crown-like structure; IR, insulin receptor; IRS-1, insulin receptor substrate 1; Glut, glucose transporter

* Corresponding author at: Division of Metabolism and Nutrition, Korea Food Research Institute, Gyeonggi-do 13539, Republic of Korea.
E-mail address: truka@kfri.re.kr (M.-S. Kim).

http://dx.doi.org/10.1016/j.jep.2016.07.023
0378-8741/

including China, Japan and Korea. Traditional medicine explains that THD improves CVD by promoting blood circulation and removing blood stagnation (Wang et al., 2012). Recently, efforts have been made to re-evaluate the efficacy of traditional medicine by obtaining biological or pharmacological evidence to serve as a scientific basis for clinical application. It was reported that THD protects neuronal cells from glutamate-induced toxicity *in vitro* (Wang et al., 2012). The beneficial effects of THD could be extrapolated to rodents or humans because muscone, one of the major compounds of THD, penetrates the blood-brain barrier (Wang et al., 2015). Here, we evaluated the *in vivo* efficacy of THD in mice with obesity-induced inflammation and a prothrombotic state as an early vascular disease model.

Obesity causes unusual metabolic inflammation. The sustained energy surplus acts as a trigger of inflammation, and the response is maintained as chronic low-grade inflammation, especially in metabolic tissues such as adipose tissue and liver (Gregor and Hotamisligil, 2011). Specifically, obesity causes the dysfunction of enlarged adipocytes and increases the infiltration of monocytes/macrophages into metabolic tissues. As a result, the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) α, interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1), increases through crosstalk between adipocytes and macrophages (Solinas and Karin, 2010). The inflammatory mediators further increase the production of pro-inflammatory cytokines in autocrine and paracrine manners, by activating transcription factors such as nuclear factor κB (NF-kB) and activator protein 1 (AP-1). Inflammatory cytokines interfere with the insulin signaling cascade by activating c-Jun N-terminal kinase (JNK) and I kappa B kinase (IKK), which in turn provokes the development of insulin resistance (Solinas and Karin, 2010). Obesity-induced adipocyte dysfunction also disturbs of adipokine secretion. In the obese state, there is decreased production of adiponectin, which has insulin-sensitizing, vascular-protective and anti-inflammatory properties, and increased release of plasminogen activator inhibitor-1 (PAI-1), which causes the prothrombotic state (Eckel et al., 2005). Adipose tissue inflammation and the change in the adipokine milieu evoke hepatic inflammation and increase the production of C-reactive protein (CRP) and fibrinogen in the liver. All these disturbances affect the vascular endothelium by stimulating the production of adhesion molecules, such as vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). Therefore, the overall condition becomes an inflammatory and prothrombotic state, which eventually develops into cardiovascular disease (Eckel et al., 2005; Endemann and Schiffrin, 2004; Kotsis et al., 2010; Van Gaal et al., 2006). Taken together, obesity itself is not a disease, but obesity-induced inflammation and the prothrombotic state are associated with important pathogenic mechanisms that are involved in the development of obesity-associated cardiovascular disease. In this study, we demonstrated the efficacy and functional mechanism of action of THD in HFD-induced obese mice showing metabolic dysregulation, inflammation, and a prothrombotic state as an early vascular model.

2. Materials and methods

2.1. THD preparation

THD was prepared by combining 8 different types of dried herbal plants at the following ratio: 4 g *Paeonia obovata* Maxim., 4 g *Cnidium officinale* Makino, 12 g *Prunus persica* (Linne) Batsch, 12 g *Carthamus tinctorius* L., 3 g *Allium fistulosum* L., 2 g *Zizyphus jujuba* var. *inermis* (Bunge) Rehder, 12 g *Zingiber officinale* Roscoe, and, 5 g *Saussurea costus* (Falc.) Lipsch. All herbal plants were purchased from Omniherb (Daegu, Korea). These materials were confirmed by Dr. Jun-Kyung Lee of Hyemin Dispensary of Oriental Medicine (Jeonju, Korea). A voucher specimen (THD) was deposited at the KM fundamental Research Division, Korea Institute of Oriental Medicine. For extraction, dried and ground herbal mixture was boiled with 10 times the weight of distilled water for 2 h at 100 °C. The extract was filtered through filter paper, and the filtrates were freeze-dried, and, stored at −70 °C. The yield was 20.02%. The THD extract was dissolved in drinking water for oral administration and DMSO *in vitro* treatment, respectively.

2.2. HPLC analysis

The chemical profile of THD was analyzed by high-performance liquid chromatography (HPLC). The reference components I (alboflorin, peoniflorin, and benzoic acid) and II (gallic acid, coumarin, cinnamic acid, and cinnamic aldehyde) were purchased from NPC Bio Technology Inc. (Daejeon, Korea). Components III (nodakenin and ferulic acid) were obtained from Wako Chemicals (Osaka, Japan). The purity of the three standard compounds was ≥98.0% by HPLC analysis. The HPLC-grade solvents, methanol, acetonitrile and water, were obtained from J.T. Baker (Phillipsburg, NJ, USA). The trifluoroacetic acid (analytical reagent grade) was procured from Sigma-Aldrich (St. Louis, MO, USA). The HPLC system consisted of a Waters Alliance 2695 system coupled with a 2998 photodiode array detector. Data processing was carried out with Empower software (Waters Co., Milford, MA, USA). The three components in THD were separated by using a Luna 5 m C18 (2) 100 A (4.6 × 250 mm, 5 μm particle size, no. 00G-4252-E0, Phenomenex Co., Torrance, CA, USA) columns. The monitored was at 230 nm for components I, 280 nm for components II and 320 nm for components III. The mobile phases were water with 0.1% (v/v) trifluoroacetic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 1.0 ml/min, with following gradient (A to B): 0–40 min, 5–60% B.

2.3. Animal experiments

Five-week-old male C57BL/6 mice were purchased from the Central Lab Animal, Inc. (Seoul, Korea), and housed two per cage in a climate-controlled environment (24 ± 1 °C at 50% relative humidity) with a 12 h light/12 h dark cycle. After adaption to this environment for 1 week, the mice were randomly divided into two groups: normal chow (NC, n=8) and high-fat diet (pre-HFD, n=15). The diets D12450B (10% calories from fat, NC) and D12492 (60% calories from fat, HFD) were purchased from Research Diets (New Brunswick, NJ, USA). The mice were fed these diets *ad libitum* with free access to water. After receiving the high-fat diet for 8 weeks, the pre-HFD group was divided into two subgroups. While continuing to eat the high-fat diet, one group was treated once daily with oral THD in 100 μl of water at a dose of 100 mg/kg body weight (HFD+THD, n=8) for 4 weeks. The freeze-dried THD powder was dissolved in drinking water every day and orally administered to mice in uniformly suspended state. For the *in vivo* efficacy evaluation study of traditional East Asian medicine as a state of decoction, the dose range of 50–200 mg/kg body weight is usually adopted (Song et al., 2016). The dose of 100 mg/kg body weight we chose is converted to approximately 2% feeding, considering the mean body weight and food consumption of mice. The other pre-HFD subgroup (HFD, n=7) and the NC group were received an oral gavage of water as a vehicle control. Total food intake and mouse body weight were measured every week and every 2 weeks, respectively. At the end of the study, the mice were fasted overnight and euthanized, and blood was collected from the orbital vein. Adipose tissue, liver, spleen, and kidney were dissected and weighed. All animal experiments were approved by the