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Lipid-lowering effects of Danhong injection on hyperlipidemia rats

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ABSTRACT

Ethnopharmacological relevance: Danhong injection (DHI), a Chinese medical product extracted from Radix et Rhizoma Salviae Miltiorrhizae (*Salvia miltiorrhiza* Bge., Labiatae, Danshen in Chinese) and Flos Carthami (*Carthamus tinctorius* L., Compositae, Honghua in Chinese), has been reported to have anti-inflammatory, anti-oxidative and anti-fibrinolytic properties and is used extensively for the clinical treatment of cardiovascular disease in clinic. This study aimed to investigate the preventive and therapeutic effects of DHI on hyperlipidemia.

Materials and methods: Forty-eight adult male Sprague-Dawley rats were randomly divided into four groups: normal control (NC), model control (MC) and DHI-treated at doses of 1.0 mL/kg and 2.0 mL/kg. The effects of DHI on serum triglyceride (TG), total cholesterol (TC), glucose, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were evaluated and insulin was determined by enzyme-linked immunosorbent assay (ELISA). Moreover, the expression of acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FAS), carnitine palmitoyl transferase 1 (CPT1), hydroxymethylglutaryl-CoA reductase (HMGR) and peroxisome proliferator-activated receptor alpha (PPAR-α) in liver were determined by real-time PCR.

Results: Compared with the MC group, rats treated with DHI had significantly reduced TG, TC, LDL-C and arteriosclerosis index (AI). Expression of FAS and HMGR mRNA was significantly reduced, whereas the CPT1 and PPAR-α were significantly increased.

Conclusion: DHI treatment was accompanied by significantly increased lipolysis in the liver and decreased fatty acid synthesis. The insights gained from this study will improve both understanding of the mechanisms involved in the effect of DHI on hyperlipidemia and the pharmacological rationale for the use of DHI in diseases caused by lipid metabolic disorders.

1. Introduction

Hyperlipidemia is a common disorder of lipid metabolism characterized by abnormal lipid levels with increased blood total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) along with decreased high density lipoprotein cholesterol (HDL-C). Hyperlipidemia is a major risk factor for the development of cardiovascular disease (Choudhary et al., 2005). Moreover, it is well-known that hyperlipidemia induced by high-fat and high cholesterol diets may be responsible for nonalcoholic fatty liver disease (NAFLD) (Vinaixa et al., 2010), which is characterized by fat deposition in the liver without alcohol abuse. Many drugs, including fibrates and statins, have rapid lipid-lowering effects and good efficacy. However, their use is limited by differences between individual hyperlipidemia patients, potential adverse effects and drug dependence (Alsheikh-Ali et al., 2004). By comparison, plant materials and their injections have minimal adverse effects and multiple targets for the prevention and cure of hyperlipidemia.

Both Danshen and Honghua are well-known traditional Chinese medicines widely prescribed for the treatment of cardiovascular disease. Because the former is cold whereas the latter is warm in nature according to traditional Chinese medical theory, these two herbs are often applied together to achieve a synergistic effect and avoid side effects, in clinical decoctions or Chinese patent medicines such as Danhong injection (DHI) (Liu et al., 2011; Zhan et al., 2008).

DHI, produced by Heze Buchang Pharmaceutical Co., Ltd. of China with drug approval number Z20026866, is a standardized Chinese materia medica extracted from Radix et Rhizoma Salviae Miltiorrhizae and Flos Carthami with a raw dose ratio of 3:1. The principal components of DHI include tanshinone, tanshinol acid and

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safflor yellow (Sun et al., 2009; Tai et al., 2005). It has been used in various diseases, especially cardiovascular disease due to its traditional Chinese medical effects of activating blood circulation, dissipating blood stasis, and dredging meridians and collaterals (Tai et al., 2005). Hyperlipidemia is a major risk factor for the development of cardiovascular disease, and numerous clinical observations have confirmed a favorable outcome in hyperlipidemia patients receiving Danshen or related pharmaceutical preparations (Li and Xu, 2008; Wang et al., 2010). However, the therapeutic effect and mechanisms of DHI in hyperlipidemia remain unclear. Thus, the present study was designed to investigate the preventive and therapeutic effects of DHI on hyperlipidemia in Sprague-Dawley (SD) rats to determine its clinical efficacy and the potential mechanisms of its effect in cardiovascular disease.

2. Materials and methods

2.1. Quality control of DHI

DHI was purchased from Shandong Buchang Pharmaceutical Co., Ltd. (Jinan, China) (drug approval number: Z20026866; product batch number: 111050). The quality control standard for DHI according to the national drugs surveillance administrative bureau is that the total amounts of danshensu ($C_9H_{10}O_5$) and protocatechuic aldehyde ($C_7H_6O_3$) should not be lower than 0.5 mg in 1 mL injection analyzed by high performance liquid chromatography (HPLC). Also, total flavonoids determined by visible light spectrophotometry should be no lower than 5.0 mg/mL against rutin ($C_{27}H_{30}O_{16}$) (He et al., 2012).

2.2. Animals

Forty-eight healthy adult male SD rats, weighing 200 ± 10 g, were obtained from the Central Animal Facility of Zhejiang Chinese Medical University (Laboratory animal certificate: scxk 20080115). All animals were cared for according to the Guide for the Care and Use of Laboratory Animals (NIH Publications, No. 80-23, revised in 1996). Housed in a room with a 12 h light–dark cycle (temperature 22–24 °C and humidity 50–60%), the rats were given ad libitum access to standard laboratory rodent chow and water. Every effort was taken to reduce the number of animals used and amount of suffering caused during the experiments, and all procedures conformed to international guidelines on the ethical use of animals.

2.3. Experimental design

Rats were fed with basic diet for 1 week in the experimental environment before the experiments were conducted. Once they had adapted to the environment, 12 rats were selected randomly as the normal control (NC) group, these were fed with basic diet, whereas the others received a high-fat diet (Xietong Medical Bioengineering Co., Ltd., Nanjing, China) comprising 68% basal diet, 10% lard, 10% yolk powder, 10% sugar and 2% cholesterol for 6 weeks. Hyperlipidemia model rats were randomly divided into three groups: model control (MC), low dose DHI (1.0 mL/kg body weight) and high dose DHI (2.0 mL/kg body weight). Each group comprised 12 rats. Once daily for 30 days, the rats received DHI, or the same volume of normal saline in the case of the NC and MC rats. At the end of the experimental period and after 12 h of fasting, the rats were anesthetized with chloral hydrate by the intra-abdominal route and sacrificed at the same time of day in all groups to avoid circadian fluctuations.

2.4. Measurement of serum lipid metabolic parameters

Blood samples were allowed to clot at 4 °C and centrifuged at 3000*g* for 15 min before harvesting the serum, serum samples were then stored at −20 °C until assayed. Liver tissues were collected, snap-frozen in liquid nitrogen and stored at −80 °C until analyzed. The blood samples were used to for the measurement of TG, TC, glucose, HDL-C and LDL-C using commercial kits (DiaSys Diagnostic Systems Co., Ltd., Shanghai, China). Arteriosclerosis index (AI) was calculated as follows: AI = (TC − HDL-C)/HDL-C (Feng et al., 2011).

2.5. Determination of serum insulin

The concentration of insulin in serum was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Bangyi Trading Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Insulin sensitivity index [ISI, −ln(fasting serum insulin × fasting serum glucose)] was calculated (Li et al., 2002).

2.6. Real-time quantitative reverse transcription PCR

Total RNA was extracted from liver using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcriptions were performed using an ImProm-II Reverse Transcription System cDNA synthesis kit. The reaction volume of 20 μL contained 0.5 μg total RNA (three replicates). The real-time PCR oligonucleotide primers (designed using Primes Premier 5.0) used for rat β-actin (internal control), acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FAS), carnitine palmitoyl transferase 1 (CPT1), hydroxymethylglutaryl-CoA reductase (HMGR) and peroxisome proliferator-activated receptor alpha (PPAR-α) are shown in Table 1. PCR was performed under standard conditions (40 cycles). All samples were analyzed in

Table 1
Oligonucleotide PCR primers.

Gene	GenBank accession number	Primers sequence (5′–3′)	Orientation	Product size (bp)
β-actin	NM_031144.3	ACTCTGTGTGGATTGGTGGC AGCTCAGTAACAGTCCGCCT	Forward reverse	137
ACC1	NM_022193.1	AACAGTGTACAGCATCGCCA CATGCCGTAGTGGTTGAGGT	Forward reverse	144
FAS	NM_017332.1	TCGACTTCAAAGGACCCAGC ACTGCACAGAGGTGTTAGGC	Forward reverse	156
PPAR-α	NM_013196.1	ATTCGGCTAAAGCTGGCGTA TGCATTGTGTGACATCCCGA	Forward reverse	123
CPTI	BC072522.1	GGACATTCCTCTCTCAGGTTTC ACCTCCTCCTTTGAACACATAC	Forward reverse	120
HMGR	NM_013134.2	TCCGTCTCCAGTCCAAAACG GTTACCACTGACCGCCAGAA	Forward reverse	125