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The metabolic change of serum lysophosphatidylcholines involved in the lipid lowering effect of triterpenes from *Alismatis rhizoma* on high-fat diet induced hyperlipidemia mice

Sen Li [a], Shuna Jin [a], Chengwu Song [a], Chang Chen [a], Yang Zhang [b], Yi Xiang [a], Yong Xu [c], Yulin Feng [d], Qian Wan [a,*], Hongliang Jiang [a,*]

[a] Tongji School of Pharmacy, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan, Hubei 430030, China
[b] Department of Pharmacy, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan, Hubei 430030, China
[c] Medical Research Center, Humanwell Healthcare (Group) Co., Ltd, 666 Gao Xin Road, Wuhan, Hubei 430075, China
[d] Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, China

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ABSTRACT

Ethnopharmacological relevance: *Alismatis rhizoma* (AR), a Traditional Chinese Medicine with lipid-regulating properties, is usually used to treat hyperlipidemia. Lysophosphatidylcholines (Lyso PCs) play a crucial role in lipid metabolism disorders. In this study, the triterpene fraction purified from boiling water extract of AR was evaluated for its lipid lowering activity using mice with high-fat diet (HFD) induced hyperlipidemia. The metabolic changes of individual Lyso PCs treated with the triterpene fraction were detected by ultra-high performance liquid chromatography–triple quadrupole-linear ion trap mass spectrometer (UHPLC–QTRAP-MS/MS).

Materials and methods: HFD induced hyperlipidemia mice were administrated with triterpene and non-triterpene fractions at doses of 180, 360 and 720 mg/kg body weight/day for 4 weeks, respectively. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and atherogenic Index (AI) in mice serum were measured. The chemical components in the lipid-lowering fraction were characterized by ultra-high performance liquid chromatography–quadrupole time of flight tandem mass spectrometry (UHPLC–QTOF-MS/MS). The changes of Lyso PC in the serum of mice treated with the lipid-lowering fraction were quantified by UHPLC–QTRAP-MS/MS.

Results: A total of 18 alisol derivatives were identified in the triterpene fraction. The hyperlipidemia mice treated with the triterpene fraction showed a significant decrease in serum TC, LDL-C and AI after continuous consumption of HFD for 4 weeks. The results also showed that 27 serum Lyso PCs in mice fed with HFD were down-regulated, and 19 were up-regulated. The abnormal serum level of Lyso PCs associated with hyperlipidemia was intervened by the alisol derivatives, with increase of unsaturated Lyso PCs and decrease of saturated ones.

Conclusions: The study demonstrated for the first time that triterpenes from the AR extract can lower serum lipid level in HFD induced hyperlipidemia mice. These metabolism changes of Lyso PCs could further improve our understanding of the potential mechanism of lipid lowering effect of AR.

1. Introduction

Alismatis rhizoma (AR) (Zexie in Chinese), the dried rhizome of *Alisma orientale* Juzepzuk (Alismataceae), are used to treat dysuria, edema, hyperlipidemia, diabetes and hypertension according to the Chinese Pharmacopoeia (2010 edition). Specifically, AR is widely used in Traditional Chinese Medicine (TCM) formulas, such as Ze Xie Tang, Dan Tian Jiang Zhi Wan and San Shen Jiang Zhi Ye, etc., as an important ingredient for treating hyperlipidemia (Commission, 2010). It was demonstrated that triterpenes are the major chemical components of AR and previous work has been

Abbreviations: AR, *Alismatis rhizome*; TCM, Traditional Chinese Medicine; Lyso PC, lysophosphatidylcholine; HFD, high-fat diet; TC, total cholesterol; AI, atherogenic index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; UHPLC–QTRAP-MS/MS, ultra-high performance liquid chromatography–triple quadrupole-linear ion trap mass spectrometer; UHPLC–QTOF-MS/MS, ultra-high performance liquid chromatography–quadrupole time of flight tandem mass spectrometry; MRM, multiple reaction monitoring; IS, internal standard; BPC, base peak chromatogram; HMG-CoA, 3-hydroxy-3-methyl glutaryl coenzyme A; LCPUFA, long-chain polyunsaturated fatty acids

* Corresponding authors.
E-mail addresses: wanqian@hust.edu.cn (Q. Wan), jianghongliang@hust.edu.cn (H. Jiang).

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mainly focus on its methanol or ethanol extract (Chen et al., 2013; Liu et al., 2010; Nakajima et al., 1994). The ethanol extract of AR has been reported to effectively decrease serum cholesterol levels (Li and Qu, 2012), protect the hepatic system (Jiang et al., 2006), and increase the urinary excretion of sodium, chloride and urea (Chen et al., 2014). However, most TCMs are prepared in the way of decoctions and administered orally. Boiling water extraction and organic solvent extraction would result in different phytochemical profiles in the extracts (Song et al., 2012). Traditionally, AR is prepared by boiling water which dissolves many water soluble constituents (proteins, amino acids and polysaccharides) along with triterpenes. However, the lipid-lowering activity of individual fraction from AR extract, such as the triterpene or the non-triterpene fraction, has not yet been studied.

Recently, metabolic profiling has attracted great interests in biomarker discovery and assessment of holistic therapeutic effects of many TCMs (Sun et al., 2012; Tan et al., 2012; Wang et al., 2012). Hyperlipidemia is a category of disorders characterized by an elevated level of lipids in the bloodstream. Lysophosphatidylcholines (Lyso PCs), which are phospholipid derivatives originating from cell membranes (Pereto et al., 2004), play an important role in atherosclerosis (Karliner, 2002), inflammatory diseases (Graler and Goetzl, 2002) and lipid metabolism disorders (Glass and Witztum, 2001). The serum Lyso PC level may serve as a more precise marker for specific metabolic phenotypes than the total lipid concentration.

In the present study, the triterpene and non-triterpene fractions were purified from boiling water extract of AR, and their lipid lowering effect was evaluated in high-fat diet (HFD) induced hyperlipidemia mice. The level of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and atherogenic index (AI) in serum were analyzed after treatment with triterpenes and non-triterpenes. Furthermore, the metabolic changes of Lyso PCs after treatment of triterpenes were detected using the targeted metabolomic approach published by our group (Jin et al., 2014). The results indicated that triterpenes from the AR extract potentially contributed to the lipid lowering effect on HFD-induced hyperlipidemia. The abnormal level of Lyso PCs associated with hyperlipidemia was intervened by the triterpenes of alisol derivatives which increased unsaturated Lyso PCs level and decreased saturated Lyso PCs level in serum. These metabolic changes of Lyso PCs could further improve our understanding of the lipid lowering effect of AR on HFD-induced hyperlipidemia.

2. Materials and methods

2.1. Plant material and chemicals

The dried rhizomes of AR were collected in Fujian Province, China and identified and authenticated by Professor Keli Chen of Hubei University of Chinese Medicine. The voucher specimens were deposited at the herbarium of Huazhong University of Science and Technology.

AB-8 macroreticular resin column was purchased from Sunresin New Materials Co., Ltd. (Xi' an, China). Simvastatin was purchased from Sanchine pharmaceutical Co., Ltd. (Hei Longjiang, China). HPLC grade acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was produced by a Milli-Q water system (Millipore, Bedford, MA, USA). Formic acid ($\geq$ 98%) of analytical grade was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Preparation of AR extracts

The dried rhizomes of AR were ground into powder (24 mesh). The powder (2 kg) was boiled twice in water (20 L) for 1 h each. The two batches were combined and filtered before loading onto AB-8 macroreticular resin column. Non-triterpenes and triterpenes were separated and eluted with 30% ethanol (15 L) and 75% ethanol (10 L), consecutively. The two fractions were concentrated on a rotatory evaporator under vacuum followed by drying in a freeze-dryer. 245.9 g of non-triterpene fraction and 44.7 g of triterpene fraction were respectively obtained, which accounts for 12.30% and 2.24% (w/w) of the dry material.

2.3. Hyperlipidemia mouse model and drug administration

Male Kunming mice (25–30 g) were purchased from Experimental Animal Center of Huazhong University of Science and Technology. The mice were housed in stainless steel cages under standard environmental conditions (23 $\pm$ 2 °C, 40–60% relative humidity, and 12 h light/12 h dark cycle) and allowed for free access to water and food. After acclimation for 3 days, all mice were randomly divided into two groups. The control group (n=8) received a standard diet, while the other groups (n=64) were fed with a HFD, which was made of standard diet (78.8%), egg yolk (10%), lard (10%), cholesterol (1%) and cholate (0.2%). After 4 weeks, blood samples were collected from caudal vein and the serum lipid level was measured. A substantial increase of serum lipid level suggested the hyperlipidemia model had been established in mice fed with HFD. These hyperlipidemia animals were then randomly divided into 8 groups of 8 mice in each group as follows in addition to the control group (C group):

C group: Normal mice fed with standard diet, administered with 0.5% carboxymethylcellulose sodium (CMC-Na) solution;

HL group: Hyperlipidemia mice fed with HFD, administered with 0.5% CMC-Na solution;

P group: Hyperlipidemia mice fed with HFD, administered with simvastatin at a dose of 20 mg/kg body weight/day;

AT_L, AT_M and AT_H group: Hyperlipidemia mice fed with HFD, administered with the triterpene fraction at a low, medium and high dose of 180, 360 and 720 mg/kg body weight/day, respectively;

N-AT_L, N-AT_M and N-AT_H group: Hyperlipidemia mice fed with HFD, administered with the non-triterpene fraction at a low, medium and high dose of 180, 360 and 720 mg/kg body weight/day, respectively.

Each group was maintained on its respective regimen for another 4 weeks. The mice were allowed for free access to water and food during the experiment. The body weight and food consumption were measured twice a week. Mice were fasted for 12 h after the last dose. On the 28th day, blood samples were collected from each mouse with ophthalmectomy under anesthesia by intraperitoneal injection of 2% sodium pentobarbital (40 mg/kg body weight). About 1–1.5 mL blood was collected from each mouse. After collection of blood, mice were euthanized by cervical dislocation under anesthesia. Serum was obtained by centrifugation at relative centrifuge force of 800g for 15 min and stored at −80 °C. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Huazhong University of Science and Technology (2014 IACUC no. 422).

2.4. Lipid level analysis

The levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in serum were analyzed by Roche Cobas 8000 modular analyzer