Toxicology and Applied Pharmacology 332 (2017) 40–51

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

UPLC-Q-TOF/MS-based urine and plasma metabonomics study on the ameliorative effects of aspirin eugenol ester in hyperlipidemia rats

Ning Ma, Isam Karam, Xi-Wang Liu, Xiao-Jun Kong, Zhe Qin, Shi-Hong Li, Zeng-Hua Jiao, Peng-Cheng Dong, Ya-Jun Yang *, Jian-Yong Li *

Key Lab of New Animal Drug Project of Gansu Province, Key Lab of Veterinary Pharmaceutical Development, Ministry of Agriculture, Lanzhou Institute of Husbandry and Pharmaceutical Science of Chinese Academy of Agricultural Sciences, Lanzhou 730050, P.R. China

ARTICLE INFO

Article history:
Received 21 February 2017
Revised 26 June 2017
Accepted 17 July 2017
Available online 18 July 2017

Keywords:
Aspirin eugenol ester
Blood lipids
Hyperlipidemia
Metabonomics
Rat

ABSTRACT

The main objective of this study was to investigate the ameliorative effects of aspirin eugenol ester (AEE) in hyperlipidemic rat. After five-week oral administration of AEE in high fat diet (HFD)-induced hyperlipidemic rats, the impact of AEE on plasma and urine metabonomics was investigated to explore the underlying mechanism by UPLC-Q-TOF/MS analysis. Blood lipid levels and histopathological changes of liver, stomach and duodenum were also evaluated after AEE treatment. Without obvious gastrointestinal (GI) side effects, AEE significantly relieved fatty degeneration of liver and reduced triglyceride (TG), low density lipoprotein (LDL) and total cholesterol (TCH) ($P < 0.01$). Clear separations of metabolic profiles were observed among control, model and AEE groups by using principal component analysis (PCA) and orthogonal partial least-squares-discriminate analysis (OPLS-DA). 16 endogenous metabolites in plasma and 18 endogenous metabolites in urine involved in glycerophospholipid metabolism, fatty acid metabolism, fatty acid beta-oxidation, amino acid metabolism, TCA cycle, sphingolipid metabolism, gut microflora and pyrimidine metabolism were considered as potential biomarkers of hyperlipidemia and be regulated by AEE administration. It might be concluded that AEE was a promising drug candidate for hyperlipidemia treatment. These findings could contribute to the understanding of action mechanisms of AEE and provide evidence for further studies.

1. Introduction

Cardiovascular disease is one of the important threats to the health of the population and cause 18 million deaths per year globally. Hyperlipidemia, one of the major risk factors for the development of cardiovascular diseases, such as coronary heart disease, myocardial infarction, cerebral stroke, atherosclerosis and hypertension, is becoming a major health problem in the world (Bowry et al., 2015). Hyperlipidemia is variably defined as a metabolic disorder disease which involves increased serum levels of triglycerides (TG), total cholesterol (TCH), low-density lipoprotein (LDL) as well as decreased levels of high-density lipoprotein (HDL) (Wasan et al., 1997).

Aspirin (acetylsalicylic acid) as a classic non-steroidal anti-inflammatory drug (NSAID) is widely used to treat inflammation and cardiovascular diseases (Nansseu and Noubiap, 2015). In clinical practice, gastrointestinal (GI) side effects are the main constraint in its application, which are mainly caused by the free acidic group and direct contact of drug with gastric mucosa (Kean and Buchanan, 2005). Chemical masking of carboxylic group in aspirin is a simple and efficient method of improving therapeutic efficacy and retarding gastrointestinal side effects (Redasani and Bari, 2012). Eugenol is a natural product and safe essential oil, which is extracted from dry alabastrum of *Eugenia caryophyllata* Thumb. It has been known for its various therapeutic effects including anti-inflammation, antibacterial, antioxidant, anti-diarrhea, and antiulcer (Capasso et al., 2000; Naidu, 1995; Yogalakshmi et al., 2010). Because of the free hydroxyl group, eugenol is structural instable and vulnerable to oxidation.

Based on the pro-drug principle, aspirin eugenol ester (AEE) was synthesized with the starting precursors of aspirin and eugenol (Li et al., 2012a). There are three advantages in AEE. Firstly, the disappearance of free hydroxyl group increases structural stability of eugenol. Secondly,

Abbreviations: AEE, aspirin eugenol ester; HFD, high fat diet; TG, triglyceride; LDL, low density lipoprotein; TCH, total cholesterol; PCA, principal component analysis; OPLS-DA, orthogonal partial least-squares-discriminate analysis; NSAID, non-steroidal anti-inflammatory drug; GI, gastrointestinal; CMC-Na, carboxymethylcellulose sodium; TIC, total ion chromatograms; LV, latent variables; CV-ANOVA, ANOVA of the cross-validated residuals; LysoPC, lysophosphatidylcholinesi; BCAA, branched chain amino acid; VIP, variance importance for projection; RT, retention time; N1MPC, N1-methyl-2-pyridone-5-carboxamide; 3ICAG, 3-indole carboxylic acid glucuronide; NAD, nicotinamide adenine dinucleotide; AA, arachidonic acid; S1P, sphingosine 1-phosphate.

* Corresponding authors at: No. 335, Jiangouyan, Qilihe District, Lanzhou 730050, P.R. China.

E-mail addresses: yangyue10224@163.com (Y.-J. Yang), lijy1971@163.com, lijianyong@caas.cn (J.-Y. Li).

http://dx.doi.org/10.1016/j.taap.2017.07.013
0041-008X/

the disappearance of carboxyl group decreases the acidity and reduces GI side effects caused by aspirin. Finally, eugenol possesses antiulcer activity based on its ability to enhance mucus production which is an important gastroprotective factor (Santin et al., 2011), and thus aspirin and eugenol play complementary roles to reduce GI damages. Therefore, AEE reduce the side effects of its precursors and improve therapeutic effect and stabilization through the disappearance of carboxyl and hydroxyl group. The acute toxicity, subchronic toxicity, teratogenicity, metabolism and pharmacodynamics of AEE had been evaluated in our previous studies, and these studies have indicated that AEE is a promising compound with good druggability (Karam et al., 2015; Li et al., 2012b, 2013b; Ma et al., 2015, 2016; Shen et al., 2015).

With the comprehensive analysis of small molecules, metabonomics provides a powerful approach to discover biomarkers in biological systems (Kell, 2006; Zhao et al., 2014). Metabonomics has been increasingly applied as a versatile tool for evaluating the toxicity and therapeutic effect of numerous compounds (Li et al., 2013a; Liu et al., 2014a). Regulation effects of AEE on blood lipids in hyperlipidemic rats has been confirmed in our previous study, in which 54 mg/kg AEE significantly reduced TG, TCH, LDL, and elevated HDL (Karam et al., 2015). However, little information is known concerning the alteration of plasma and urine metabonomics associated with AEE therapeutic effects. With the application of UPLC-Q-TOF/MS analysis, the objective of this study was to find out more evidences to understand and illustrate the possible underlying mechanism of AEE against hyperlipidemia. Moreover, the blood lipid-lowering effects and GI toxicity of AEE were also assessed in this study.

2. Materials and methods

2.1. Reagents and materials

AEE (transparent crystal, purity: 99.5% with RP-HPLC) was prepared in Key Lab of New Animal Drug Project of Gansu Province, Key Lab of Veterinary Pharmaceutical Development of Agricultural Ministry, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS. Carboxymethylcellulose sodium (CMC-Na) was supplied by Tianjin Chemical Reagent Company (Tianjin, China). MS-grade formic acid was supplied by TCI (Shanghai, China). Deionized water (18 MΩ) was prepared with a Direct-Q®3 system (Millipore, USA). MS-grade acetonitrile was purchased from Thermo Fisher Scientific (USA). The TG, TCH, LDL and HDL kits were provided by Ningbo Medical System Biotechnology Co., Ltd. (Ningbo, China). Standard compressed rat feed and high diet feed (HFD) were supplied by Keao Xieli Feed Co., Ltd. (Beijing, China). Standard rat diet consisted of 12.3% lipids, 63.3% carbohydrates, and 24.4% proteins (kcal) and HFD (77.8% standard diet, 10% yolk power, 10% lard, 2% cholesterol and 0.2% bile salts) consisted of 41.5% lipids, 40.2% carbohydrates, and 18.3% proteins (kcal). Erba XL-640 analyzer (German) was used to measure blood lipid levels.

2.2. Animals

Male Sprague-Dawley rats, aged 6 weeks and weighing 165–180 g, were purchased from Gansu University of Chinese Medicine (Lanzhou, China). Rats were housed in plastic cages (size: 50 × 35 × 20 cm, 10 rats per cage) with stainless steel wire cover and chopped bedding. Rat feed and drinking water were supplied *ad libitum*. Light/dark regimen was 12/12 h and living temperature was 22 ± 2 °C with relative humidity of 55 ± 10%. Animals were allowed a 2-week quarantine and acclimation period prior to start of the study. Animal welfare and experimental procedures were performed strictly in accordance with the Guidelines for the Care and Use of Laboratory Animals. All of the experimental protocols and procedures were approved by the Institutional Animal Care and Use Committee of Lanzhou Institute of Husbandry and Pharmaceutical Science of Chinese Academy of Agricultural Sciences.

2.3. Drug preparation

AEE was ground and its suspension was prepared in 0.5% CMC-Na.

2.4. Study design

Fig. 1 showed the study design of this experiment. Rats were randomly separated into two groups. Group I as control group received standard diet ($n = 10$). Group II as model group received high fat diet (HFD) ($n = 20$). The blood lipid levels were examined after HFD administered for 8 weeks, and the results indicated that the hyperlipidemia disease was established successfully (Table A.1). After that, Group II was divided into two groups including model and AEE groups. Based on individual weekly body weight, AEE was intragastrically administrated at the dosage of 54 mg/kg. The rats in control and model groups were received equal volume of 0.5% CMC-Na as AEE group. The administration time of AEE was five weeks and HFD was continuously fed during the experiment period.

2.5. Sample collection

Rats were fasted for 10–12 h before blood sampling. At the end of 8th week, rats were euthanized with 10% chloral hydrate and then the blood samples (1–1.5 mL) were withdrawn from the tip of the tail into vacuum tubes to obtain serum samples (4000 ×*g*, 4 °C for 10 min). The

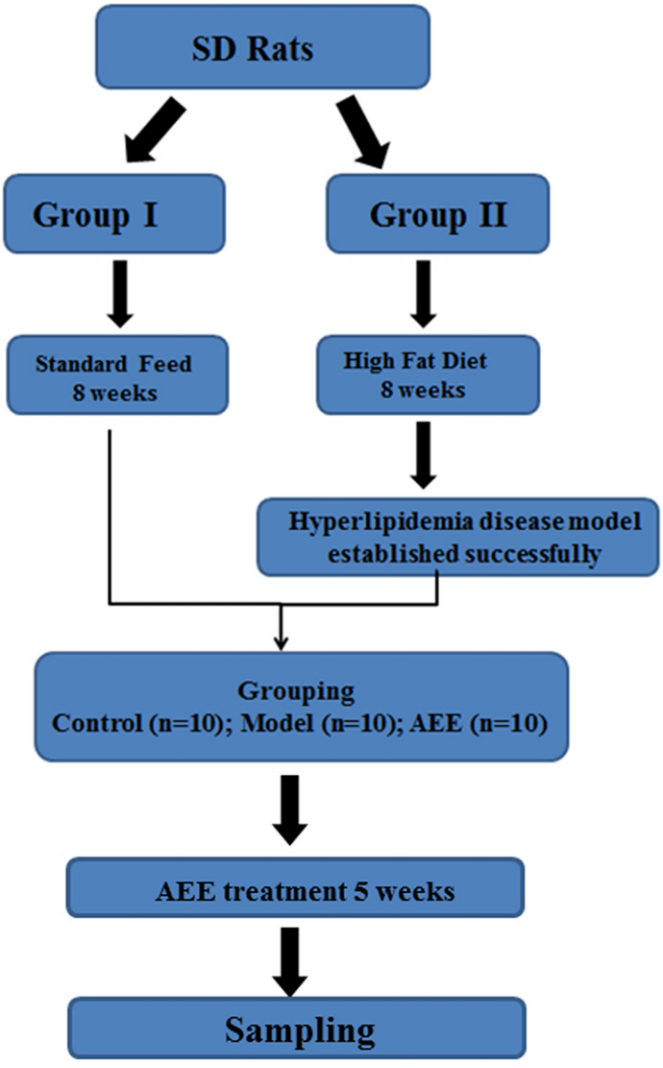

Fig. 1. Study design of the experiment.