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Metabolomic study on the faecal extracts of atherosclerosis mice and its application in a Traditional Chinese Medicine

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ABSTRACT

The intestinal microbiota and their metabolites are closely related to the formation of atherosclerosis (AS). In this study, a metabolomic approach based on the reversed-phase liquid chromatography/quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) platform was established to analyze the metabolic profiling of fecal extracts from AS mice model. The established metabolomic platform was also used for clearing the effective mechanism of a Traditional Chinese Medicine (TCM) named Sishen granule (SSKL). Totally, sixteen potential biomarkers in faeces of AS mice were identified and 5 of them could be reversed by SSKL. Through functional analysis of these biomarkers and the established network, lipid metabolism, cholesterol metabolism, energy cycle, and inflammation reaction were considered as the most relevant pathological changes in gastrointestinal tract of AS mice. The metabolomic study not only revealed the potential biomarkers in AS mice' faeces but also supplied a systematic view of the pathological changes in gastrointestinal metabolite in AS mice. This metabolomic study also demonstrated that SSKL had the therapeutic effectiveness on AS through partly reversing the lipid metabolism, inflammation and energy metabolism.

1. Introduction

Atherosclerosis (AS) is the main cause of cardiovascular disease with a process of progressive intimal thickening of the arterial wall [1]. AS is a multi factorial disease. Several risk factors such as cigarette smoking, hypercholesterolemia, hypertension, hyperglycemia and work stress have been demonstrated to be closely related to the processing of AS. Many compelling hypotheses about the pathophysiology of atherosclerosis have been revealed such as lipoprotein oxidation, inflammation and immunity [2]. Yet, despite these steady progress, we still lack complete evidence to show the mechanism of AS.

In recent years, the importance of metabolites' effects on AS formation has been identified. For example, both bisphenol A (BPA) and vitamin D had been demonstrated that their circulating levels were related to atherosclerosis [3,4]. Some metabolomic methods were also developed and provided new insight into the pathophysiology of AS [5–8]. The analyzed samples included serum, plasma, urine and atrial tissue. Some biomarkers such as 4-hydroxyproline, betaine and dimethylglycine were successively identified in AS formation process [9,10]. These biomarkers' exploration improved risk stratification for subclinical atherosclerosis in comparison to conventional lipids and could potentially be useful for early cardiovascular risk assessment.

On the other side, it has been demonstrated that the intestinal microbiota and their metabolites were also closely related to the formation of AS. Wang et al. demonstrated a unique cluster

Abbreviations: AS, atherosclerosis; LC-Q-TOF-MS, liquid chromatography/quadrupole time-of-flight mass spectrometry; SSKL, sishen granule; TCM, Traditional Chinese Medicine; BPA, bisphenol A; HPLC, high pressure liquid chromatography; TC, total cholesterol; TG, total triglycerides; LDL, low density lipoprotein receptor; LC, liquid chromatography; ESI, electrospray ionization; PCA, principal component analysis; PLS-DA, partial least-squares discriminant analysis; VIP, values of variable importance projection; ANOVAs, one-way analyses of variance; AA, arachidonic acid; SCFAs, short chain fatty acids; EA, eicosapentaenoic acid; VSMC, vascular smooth muscle cell; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor.

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of three phospholipid-associated molecules that appear to promote AS [11]. Robert et al. demonstrated that chronic dietary L-carnitine supplementation in mice altered cecal microbial composition and increased AS [12]. The study of a relationship between gut-flora-dependent metabolites and AS pathology will provide opportunities for the development of therapeutic approaches for AS.

So, to reveal the pathological process of AS in gastrointestinal metabolites level and supply the potential treatment method for clinic, a metabolomic platform was setup and applied based on high pressure liquid chromatography-quadrupole time-of-flight mass spectrometry (HPLC-Q-TOF-MS). It was the first time to comprehensively analyze the gastrointestinal metabolites in faeces in AS mice by using HPLC-Q-TOF-MS platform. Furthermore, we also studied the effectiveness of a traditional Chinese medicine named SSKL (consists of four medicinal materials including *Radix pseudostellariae*, *Salvia miltiorrhiza*, *Radix adenophorae* and *Radix sophorae flavescentis*) on AS and revealed the mechanism of SSKL in metabolite level using the established metabolomic platform.

2. Experiment

2.1. Materials

Formic acid (MS grade) was purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile and methanol (MS grade) used for purposes of MS analysis were purchased from J. T. Baker (Phillipsburg, NJ). Ultrapure water was prepared using a Milli-Q water purification system (Millipore). Commercial standards were purchased from Sigma–Aldrich (MO, USA). SSKL was kindly offered by the branch of Shanghai First People's Hospital (Shanghai, China). The assay kits for detecting total cholesterol (TC) and total triglycerides (TG) were purchased from Nanjing Jiancheng Bio-engineering Institute (Nanjing, China).

2.2. Animal model and drug administration

Twenty six male mice (fifteen-week-old) with low density lipoprotein receptor (LDLR) deletion were purchased from the Slac Laboratory Animal Co., Ltd. (Shanghai, China). All mice were housed in metabolic cages of stainless steel and randomly divided into three groups (control, AS and SSKL treatment groups) in a room that was lit from 6:00 AM to 6:00 PM and kept at 25 °C. All diets and water were provided ad libitum. Two kind of diets including standard rodent diet (Slac Laboratory Animal Co., Ltd., China) and high fat diet (Jiangsu medicience pharmaceuticals company, China) with 4% cholesterol, 1% sodium cholate, 0.5% propylthiouracil, 10% lards and 84.5% basic diet were used in this study.

First, standard rodent diet was fed for all mice for 3 week. Within the next 12-week experimental period, the control group (6 mice) was continued on the standard diet and the AS group (10 mice) and SSKL treatment group (10 mice) were fed high fat diet. The SSKL treatment group was orally administrated SSKL solution addition-

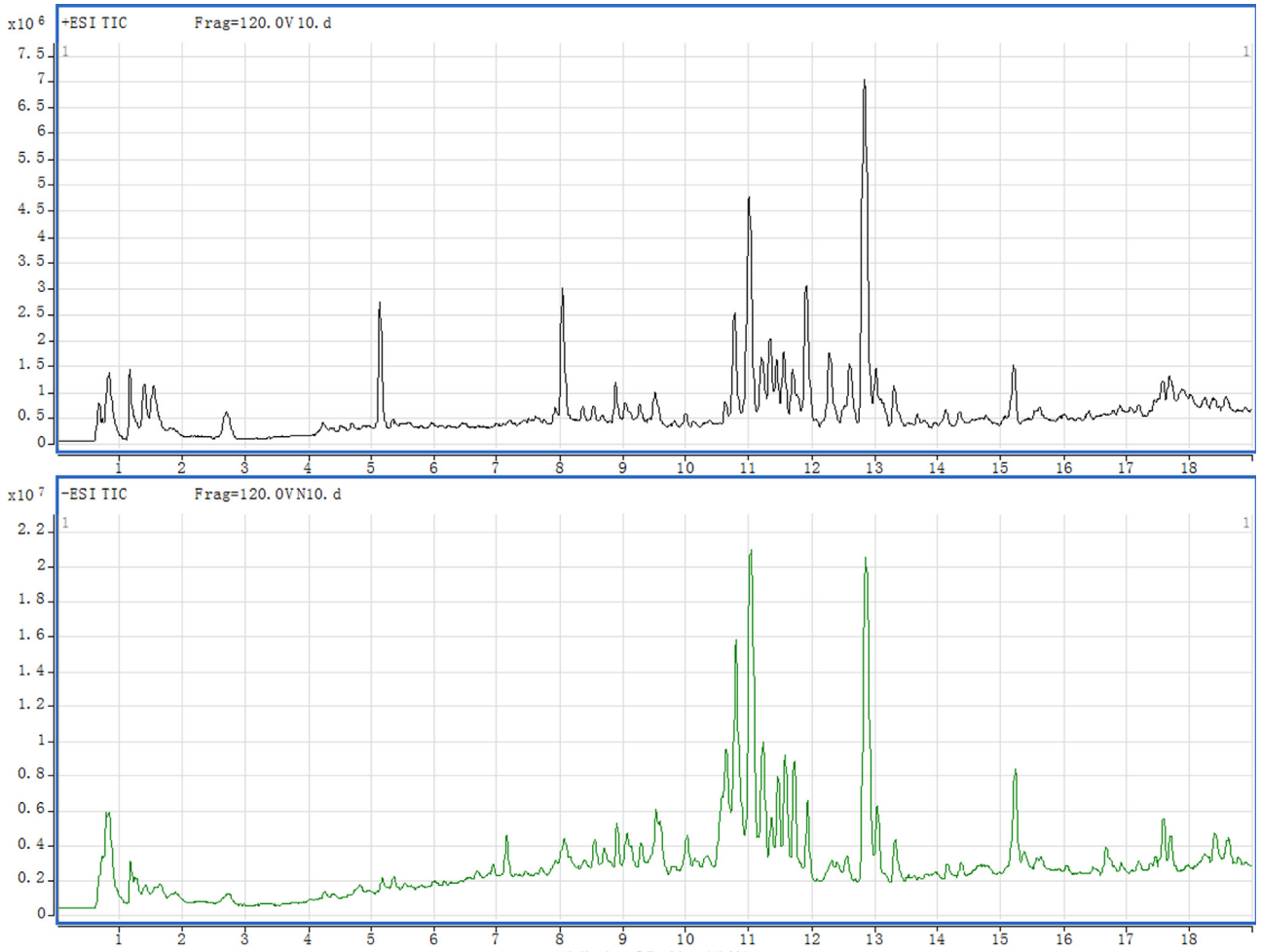

Fig. 1. The typical chromatograms of faeces sample after optimization procedure. (A) Represents the positive mode and (B) represents the negative mode.