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Aberrant purine metabolism in allergic asthma revealed by plasma metabolomics

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ABSTRACT

Asthma is a disease characterized by chronic relapsing airways, and its etiology remains incompletely understood. To better understand the metabolic phenotypes of asthma, we investigated a plasma metabolic signature associated with allergic asthma in ovalbumin (OVA)-sensitized mice by using ultra high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS). Sixteen metabolites were characterized as potential pathological biomarkers related to asthma. Among them, 6 (dodecanoic acid (**P1**), myristic acid (**P2**), phytosphingosine (**P3**), sphinganine (**P4**), inosine (**P13**) and taurocholic acid (**P15**)) were first reported to have potential relevance in the pathogenesis of experimental asthma. The identified potential biomarkers were involved in 6 metabolic pathways and achieved the most entire metabolome contributing to the formation of allergic asthma. Purine metabolism was the most prominently influenced in OVA-induced asthma mice according to the metabolic pathway analysis (MetPA), suggesting that significantly changes in inflammatory responses in the pathophysiologic process of asthma. The metabolites of purine metabolism, especially uric acid (**P12**) and inosine (**P13**), may denote their potential as targeted biomarkers related to experimental asthma. The decreased plasma uric acid (**P12**) suggested that inflammation responses of allergic asthma inhibited the activity of xanthine oxidase in purine metabolism, and manifested the severity of asthma exacerbation. The increased level of inosine (**P13**) suggests that inflammatory cells induce adenosine triphosphate (ATP) breakdown, resulting in excessive expression of adenosine deaminase (ADA) in the formation of allergic asthma. These findings provided a novel perspective on the metabolites signatures related to allergic asthma, which provided us with new insights into the pathogenesis of asthma, and the discovery of targets for clinical diagnosis and treatment.

1. Introduction

Asthma is a prevalent and serious chronic inflammatory airway disease with reversible airway obstruction and airway hyperresponsiveness. Despite remarkable advances in diagnosis and long-term management, asthma remains a serious public health concern, affecting an estimated 300 million people worldwide [1,2]. Although there is evidence to suggest that cytokines (TNF-α, IL-13, IL-4), endogenous reactive oxygen species (ROS), and reactive nitrogen species ([RNS] such as hydrogen peroxide, superoxide, and nitrous oxide [NO]) are responsible for the inflammation and tissue damage resulting from asthma [3]. Incomplete understanding of asthma's etiology and biological mechanisms are major impediments in preventing and treating this disease.

As a complex clinical syndrome with multiple genetic and environmental factors contributing to its phenotypic expression, new integrated systems approaches are needed to investigate the pathogenic mechanisms of asthma. Metabolomics, is impressive and deals with the comprehensive analysis of endogenous compounds and their dynamic changes, caused by a range of inherent and external factors. In addition, metabolomics has been employed in many fields including the diagnosis and treatment of diseases, biomarker discovery, and exploration of pathogenesis [4,5]. There-

Abbreviations: OVA, ovalbumin; UPLC/MS, ultra performance liquid chromatography/mass spectrometry; MetPA, metabolic pathway analysis; ATP, adenosine triphosphate; ADA, adenosine deaminase; BALF, bronchoalveolar lavage fluid; HE, hematoxylin-eosin; QC, quality control; RT, retention time; BPI, base peak intensity; PCA, principal cmponents analysis; OPLS-DA, orthogonal to partial least squares-discriminate analysis; VIP, variable importance of project; AMP, adenosine monophosphate; LysoPC, lysophosphatidylcholine; PS, phosphatidylserine; SPP, sphingosine 1-phosphate; EBC, exhaled breath condensate; HDM, house dust mite.

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fore, utilizing a metabolomics platform to explore disease-relevant metabolic profile changes and molecular signatures of disease processes can provide valuable information for a deeper understanding of the pathological mechanisms of disease and drug treatment, especially in complex diseases.

The mouse model of OVA-induced allergic asthma replicates many of the features of human asthma, including airway hyperreactivity and airway inflammation. Therefore, it has been often used to elucidate asthma pathology and evaluate new therapeutic agents. Metabolomic analysis of bronchoalveolar lavage fluid (BALF) and lung metabolite profile in OVA-induced allergic asthma have uncovered a panel of potential disease-related metabolic biomarkers and several metabolic pathway changes in experimental asthma [6]. While the results to date are promising, the collection of BALF and lung samples have their major disadvantages, which include invasiveness and potential risks of complications. By contrast, plasma can be collected at minimal risk, and plasma incorporate the functions and phenotypes of many different parts of the body in a single sample [7]. Therefore, plasma is very suitable for unveiling the metabolic alterations associated with asthma.

In the present study, UPLC-Q-TOF/MS based metabolomics was applied to investigate the metabolic profiles of plasma and potential biomarkers in a mouse model of OVA-induced allergic asthma; the purpose of which was to facilitate understanding of the biological mechanism of allergic asthma and to aid clinical diagnosis and treatment.

2. Experimental

2.1. Reagents and materials

HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). The water used for UPLC was purified by a Milli-Q system from Millipore (Billerica, USA). Formic acid (HPLC grade) was obtained from Tedia (Fairfield, USA). Uric acid, Inosine, L-tryptophan, Taurocholic acid, OVA, Ammonium formate, Aluminium hydroxide ($Al(OH)_3$) and Leucine-enkephalin were purchased from Sigma Aldrich (St. Louis, USA). All other used chemicals were of analytical grade.

2.2. Experimental design

Sixteen healthy, female BALB/c mice, 4–6 weeks old were purchased from the Institute of Laboratory Animal Science, CAMS & PUMC (Beijing, China). The mice were housed in cages for one week to adapt to the environment under controlled conditions of 12 h light-12 h dark cycles (lights on from 6:00 a.m.–6:00 p.m.), 40–60% relative humidity and temperature (20–25 °C) with commercial diet and water available *ad libium*. All experimental procedures were approved by the Ethics Committee of the Institute of Medicinal Plant Development, CAMS & PUMC.

The animals were randomly divided into two groups. The model mice were sensitized and challenged with OVA to develop allergic airway inflammation [8]. Briefly, mice were sensitized by intraperitoneal (i.p.) injections of 10 μg OVA and 4 mg $Al(OH)_3$ suspended in 0.2 mL saline on days 0, 7, and 14. From days 16–22, mice were challenged with 4% OVA aerosol for 30 min. Saline aerosol was used as a negative control.

2.3. Sample collection

Mice were anesthetized 24 h after the last aerosol challenge. The blood samples were collected in a standard protocol with sodium heparin as anticoagulant and centrifuged at 4000 rpm for 15 min at 4.0 °C. The supernatants were stored at −80 °C for metabolomics study.

The lung tissues were quickly removed after blood collected. The middle lobe of right lung was cut and put into a flasket containing 10% buffered formalin solution for the pathological analysis.

2.4. Pathology

Lung tissues from control group and model group were subjected to pathological observations. The middle lobe of right lung from each mice was fixed in 10% buffered formalin solution for 48 h, embedded in paraffin, 5 μm sectioned, and stained with hematoxylin-eosin (HE). Images were obtained and studied under light microscopy (Olympus Corp., Tokyo, Japan).

2.5. Plasma metabolomics

2.5.1. Sample preparation

Plasma (200 μL) was added into acetonitrile (800 μL), vortex-mixing for 30 s, and centrifuged at 4000 rpm for 15 min to precipitate the proteins. 750 μL of protein free supernatant was collected and dried with nitrogen at 37 °C. The dried residue was reconstituted in 100 μL of acetonitrile-water (1:1, v/v), after centrifugation for 15 min at 13,000 rpm, an aliquot of 2 μL was injected for UPLC/MS analysis.

2.5.2. Method development and validation

The plasma samples were analyzed on a Waters Acquity™ Ultra Performance LC system (Waters Corp., Milford, USA) equipped with a BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters Corp., Milford, USA). The flow rate was 0.45 mL/min, the autosampler temperature was kept at 4 °C, the column compartment was set at 40 °C, and the total separation time for both ionization modes was 20 min. The mobile phase was composed of solvents A (2 mM ammonium formate in 95% H_2O/5% acetonitrile + 0.1% formic acid) and B (2 mM ammonium formate in 95% acetonitrile/5% H_2O + 0.1% formic acid). The gradient program was optimized as follows: 0–0.5 min, 1% B; 0.5–2 min, 1–45% B; 2–10 min, 45–70% B; 10–14 min, 70–100% B; 14–17 min, washing with 100% B, and 17–20 min, equilibration with 1% B. The eluent from the column was directed to the mass spectrometer without split.

A Waters SYNAPT G2HDMS (Waters Corp., Manchester, UK) was used to carry out the mass spectrometry with an electrospray ionization source (ESI) operating in positive and negative ion mode. The parameters were set as previously described [9]. The capillary voltages were set at 3.0 and 2.5 kV, sample cone voltage 40 V, extraction cone voltage 4.0 V, respectively. Used drying gas nitrogen, the desolvation gas rate was set to 800 L/h at 450 °C, the cone gas rate at 40 L/h, and the source temperature at 120 °C. The scan time and inter scan delay were set to 0.15 and 0.02 s, respectively. Leucine-enkephalin was used as the lockmass in all analyses (m/z 556.2771 for positive ion mode and m/z 554.2615 for negative ion mode) at a concentration of 0.5 μg/mL with a flow rate of 5 μL/min. Data was collected in centroid mode from m/z 100 to m/z 1500.

To ensure the stability of sequence analysis, a quality control (QC) sample was prepared by pooling the same volume (10 μL) from each plasma sample and then preparing the pooled QC sample in the same way as the samples. The pooled QC sample was analyzed randomly through the analytical batch. The extracted ion chromatographic peaks of ten ions were selected for method validation. The repeatability of the method was evaluated using 6 replicates of the QC sample. The precision of the injection was assessed using 6 replicated analyses of the same plasma sample. The relative standard deviations (R.S.D%) of the retention time and m/z are shown in Table S1 .