Application of Liquid Chromatography-Mass Spectrometry-Based Metabolomics in Cell Culture, Drug Study, and Diseases

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Abstract: Metabolomics, the global study of metabolites within cells, biofluids, tissues or organisms, has drawn people's attention in the recent years. As one of the most widely used analytical methods, Liquid Chromatography-Mass Spectrometry (LC-MS) is commonly used to perform metabolomic analysis. Suitable sample preparation method is needed prior LC separation. It is best to use minimal sample preparation for metabolomics analysis or at least as little as one can get away with considering the purposes of metabolome profiling. Next, identification and quantification of significantly changed metabolites are carried out, with the aim of correlating their changes with pathological states, or with the effect of external influencing factors such as drugs or contaminants. Applications of metabolomics are also presented in this paper through a number of published researches using such methods in cell culture, drug study, and diseases. Overall, we provide an objective description of the LC-MS-based metabolomics, and summarize its application in cell culture, drug study, and diseases.

Key words: Metabolomics, liquid chromatography-mass spectrometry, cell culture, drug study, disease.

1. Introduction

Metabolomics analysis of all small-molecule metabolites in organisms is an emerging omics technology alongside genomics and proteomics. Over the past decade, metabolomic study has accelerated [1]. By combining liquid chromatography-mass spectrometry (LC-MS) with multivariate statistical analysis, it particularly conducive to identifying pathophysiologically affected processes and to elucidate novel physiological and pathological mechanisms using metabolomic strategies [2]. Metabolomics has unique advantages over genomics, transcriptomics, and proteomics. Metabolites are end-products that are produced downstream of gene and protein activity, and their composition and content directly affect the phenotype [3], [4]. Hence, changes in the metabolome are rapid and represent the final response of an organism to both internal and external stimuli.

Metabolome profiling is typically performed either by targeted or untargeted methods. Targeted metabolomics focus on accurate identification and quantitation of a defined set of metabolites in biological samples, which are typically predetermined by the scientific question at hand [5]. While untargeted metabolomics is to qualitatively and quantitatively analyze all metabolites within biological samples [6]. The resulting data sets are then uploaded into statistical data analysis tools to find the significantly changed metabolites (Fold change >1.5, $p<0.05$). Analysis of positional enrichments reveals activity of alternative metabolic pathways contributing to the group separation between control and phenotype of interest [7].

Herein, we discuss the application of LC-MS-based metabolomics in cell culture, drug study, and diseases. This review includes four parts: 1. LC-MS based metabolomics; 2. application of LC-MS based metabolomics in cell culture; 3. LC-MS based metabolomics in the study of drugs; and 4. application of LC-MS based metabolomics in diseases. Further research could be conducted via the combination of targeted metabolomics with untargeted metabolomics for deep understanding of the underlying research questions.

2. Liquid Chromatography-Mass Spectrometry Based Metabolomics

Metabolomics is the "global" study of metabolite changes in a biological system. Generally speaking, metabolomic study includes three steps: sample preparation, instrument analysis, and data interpretation. When designing an experiment for metabolomics, the first step is to extract metabolites from a specimen, liquid extraction (methanol extraction) is one of the commonly used methods. Sample is then collected in a vial and the vial is put in a rotator in order to get the supernatant. As an example, Liang Cui *et al.* [8] used methanol extraction for the study of serum samples. In their study, 50 μL serum samples were thawed at 4 °C and serum proteins were precipitated with 200 mL ice-cold methanol. After vortexing, the mixture was centrifuged and the supernatant was collected, dried, and analyzed by LC-MS. Grounded coffee samples were studied by Raquel Pérez-Míguez. The solid-liquid extraction procedure was also performed using methanol extraction [9]. It is not very common to compare different sample treatment methods. Interestingly, wine samples were tested by three non-specific pre-treatments: centrifugation, filtration, and direct injection [10]. Results indicate that fewer compounds appeared when syringe filters were used. In addition, the repeatability experiments showed that the best repeatability was obtained via centrifugation, so centrifugation for 5 min was chosen as the best pretreatment condition.

LC-MS combines the physical separation capabilities of LC with the mass analysis capabilities of MS, which is one of the most widely used analytical platforms for metabolomics [11]. During LC separation, a mobile phase carries the sample through a stationary phase (column). Substances with different physical and chemical properties have different interaction between the mobile and stationary phases, and thus are separated [12]. MS is mainly composed of sample introduction, ionization source, mass analyzer, and detector. Samples are introduced by LC system, the analytes are then ionized in the ionization source for further mass analysis, by which the chemicals will be determined based on the different mass-to-charge ratio [13]. Both positive and negative modes are used for the analysis to indicate what molecules are present. The chromatograms would show different characteristics between the control group and the sample group (Fig. 1). Lastly, data analysis should be performed in order to determine the significantly changed metabolites. Data analyses are conducted via software, e.g. Mass Profiler Professional (MPP) software. When analyzing data, the measurements indicate what molecules are present. There should be a list of the metabolites that are significantly changed between the control and sample groups.

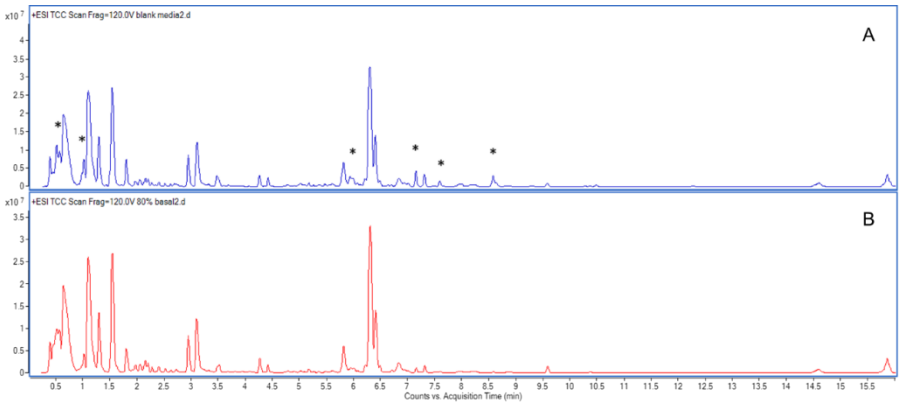

Fig. 1. Typical LC-MS chromatograms of control (A) and sample (B), the different characteristics are labeled by asterisk [14].

3. Application of LC-MS Based Metabolomics in Cell Culture

Metabolomics in science benefits many ways. However, the potential of metabolomics in traditional and emerging areas in cell culture has yet to be realized. The diverse potential of metabolomics in many fields, including cell engineering, has made it a universal tool for industrial, medical, and research purposes [15]. For example, Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. Till today, the gold standard treatment for PD is levodopa. However, levodopa is metabolized by catechol-O-methyltransferase (COMT) to 3-O-methyldopa in human CNS and loss effects. To minimize this problem, selective COMT inhibitors are developed. Tolcapone is one of the most widely used COMT inhibitors. The effects and fate of tolcapone in the human central nervous system (CNS) are still not very clear since human brain study is limited by ethical and practical considerations. Tannenbaum *et al.* have developed a human-on-a-chip system and investigated tolcapone metabolism and brain metabolomics [16]. First, the metabolic fate of tolcapone in the human-on-a-chip system was studied. Three new tolcapone metabolites were reported by LC-MS analysis, and MS/MS confirmation. Furthermore, metabolomics has been carried out in the brain microphysiological system. The results revealed that tolcapone treatment affected the endogenous metabolic pathways including the tryptophan and phenylalanine metabolism, glycerophospholipid metabolism, energy metabolism, and aspartate metabolism.

The growth characteristics of cell culture processes are also evaluated. Sang Bong Lee and co-authors used both GC-MS and LC-MS methods to conduct metabolite profiles of *Lactobacillus sakei* and its growth media, based on different culture times (0, 6, 12, and 24 h). The alteration of cell and media metabolites during cell growth was strongly associated with energy production, suggesting that MS-based metabolomic analysis might be a useful tool for understanding the growth characteristics of cells [17].

4. LC-MS Based Metabolomics in the Study of Drugs

Metabolomics is also a great way to test out drugs, and to help us find the effects of drugs on the human body. In such a study, a control group without taking the drug and a sample group after taking the drugs are analyzed by LC-MS. Statistical analysis is further applied in the determination of significantly changed metabolites during drug treatment. Lastly, pathway analysis is used to find the endogenous pathways perturbed by drug dosing. Metabolic networks associated with serotonin deficiency were investigated by LC-MS based metabolomics [4]. Serotonin is an important neurotransmitter, and serotonin deficiency has been associated with multiple pathological conditions such as Alzheimer's disease and Parkinson's disease. In this study, the serotonin deficiency was achieved through pharmacological inhibition of tryptophan hydroxylase (Tph) using the drug of p-chlorophenylalanine (pCPA). Serotonin levels were significantly reduced in both the brains and serum of the pCPA-treated mice compared with control mice (Fig. 2). Next, a metabolomics approach was employed using UPLC-QTof-MS to identify potential biomarkers perturbed by the pharmacologically induced serotonin deficiency in the pCPA-treated mice. 21 endogenous metabolites were found to be significantly changed (FC >1.5, $P<0.05$), which are associated with amino acid, energy, purine, lipid and gut microflora metabolisms.

The influences of the traditional Chinese medicine (TCM) are also evaluated by metabolomics. For example, Hongyan Dong and co-authors used spontaneously hypertensive rats with liver-yang hyperactivity syndrome as model animals to study the mechanism of the therapeutic of hypertension by Tianma Gouteng Decoction (TGD) [18]. Results show that TGD treatment mediated 15 biomarkers by regulating metabolisms of glycerol phospholipid, sphingomyelin, energy and amino acid (Fig. 3).

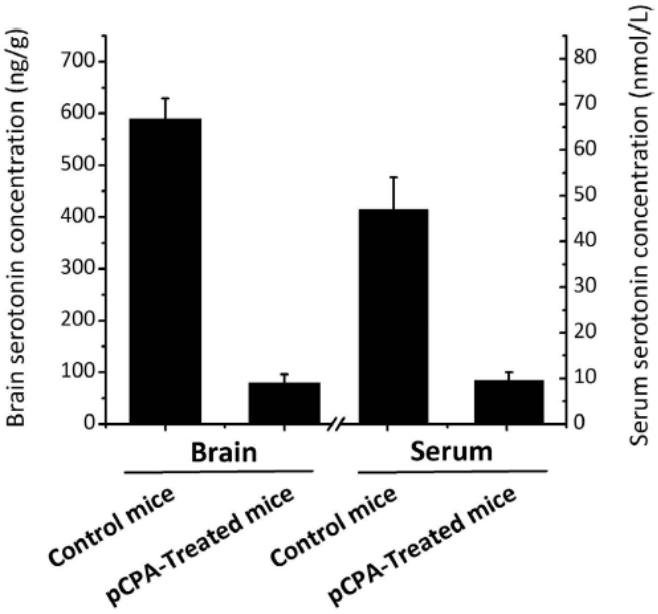

Fig. 2. Brain and serum serotonin concentrations of the control mics and pCPA-treated mice [4].

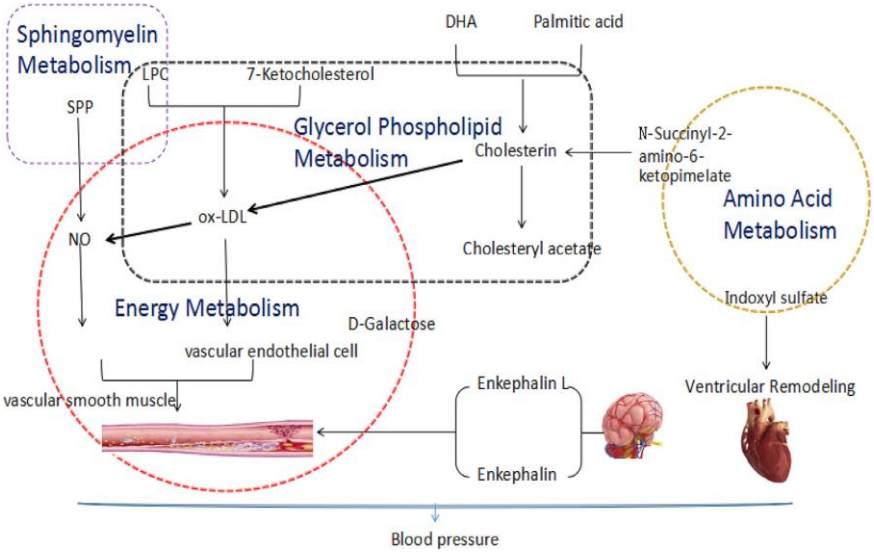

Fig. 3. The mechanism map of potential pharmacological biomarkers of TGD [18].

5. Application of LC-MS Based Metabolomics in Diseases

When applying to diseases, metabolomics gives an understanding of how diseases can be diagnosed, identifying biomarkers, and most importantly help to find a potential mechanism. In order to study the pathophysiological changes in Alzheimer's Disease (AD), Eugenia Trushina's group applied a LC-MS-based non-targeted metabolomics approach to determine global metabolic changes in plasma and cerebrospinal fluid from the same individuals with different AD severity [19]. 342 plasma and 351 CSF metabolites were found to be significantly altered, indicating that energy metabolism, Krebs cycle, mitochondrial function, neurotransmitter and amino acid metabolism, and lipid biosynthesis were perturbed by AD. This study could advance our understanding of the early disease mechanisms shared in progression from cognitively normal to AD.

Metabolomics is useful for understanding the Inflammatory Bowel Disease (IBD). Recombinase-activating gene-2-deficient (Rag2−/−) mice infected with Helicobacter hepaticus (*H. hepaticus*) have been developed as

an animal model to imitate naturally occurring inflammatory events. The control group was the normal mice that were not infected. Metabolomic analysis revealed that *H. hepaticus* infected mice dramatically changed numerous metabolite pathways, including tryptophan metabolism, glycerophospholipids, methionine-homocysteine cycle, citrate cycle, fatty acid metabolism and purine metabolism, with the majority of metabolites being down-regulated. In particular, there were notable effects of gut microflora on the blood metabolites in infected animals. Since there was a noticeable difference between the control group and sample group, it was concluded that the unique chemical pathways were the cause of the IBD [14].

6. Conclusions

Overall metabolomics in the realm of science and medication has helped with understanding more about chemistry and biology in many ways. Metabolomics now becomes a very important strategy into the study of biology system through molecular level; By using LC-MS method, scientists can now analyze metabolites in the cell samples, tissue samples or biofluids, making it simpler than ever to figure out the significantly changed metabolites and pathways. The application in the healthcare system is also beneficial as it allows people to conduct drug test, find biomarkers of diseases, and even understand the mechanism for diseases by metabolomic analysis. Metabolomics has helped society in many ways today, and hopefully benefit humanity in the future, to progress the cure for many current incurable diseases. The metabolomics field is relying on methodological advancements. Novel technologies for rapid, sensitive, and matrix-free analysis will open a new window for future study.

Conflict of Interest

There is no conflict of interest in this paper.

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