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RESEARCH PAPER

Study on Tissue Distribution of A Variety of Endogenous Metabolites By Air Flow Assisted Ionization-Ultra High Resolution Mass Spectrometry Imaging

WANG Zhong-Hua[1], HE Bing-Shu[1], SUN Cheng-Long[2], SONG Xiao-Wei[2], HE Jiu-Ming[2], ZHANG Rui-Ping[2], ABLIZ Zeper[1,2,*]

[1] Centre for Imaging & Systems Biology, College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China
[2] State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

Abstract: As a promising new molecular imaging technique, mass spectrometry imaging (MSI) has attracted more and more attention in the field of biomedicine. A method of air flow assisted ionization-ultra high resolution mass spectrometry-based mass spectrometric imaging (AFAI-MSI) was developed to profile endogenous metabolites in rat kidney tissue in this study. Rat kidneys were collected and cut into frozen tissue sections, and then were analyzed on an AFAI-MSI system operated in positive ion mode using acetonitrile-isopropanol-water (4:4:2, *V*/*V*, 5 μL min^{-1}) as spray solvent, nitrogen gas as spray gas (0.6 MPa) and air as assisting gas (45 L min^{-1}). The mass range and resolution were set to be 70–1000 Da and 70000, respectively. As a result, a total of 38 metabolites, ranging from 10^3 to 10^7 in signal strength and belonging to different metabolite types, including organic amines, sugars, vitamins, peptides, neurotransmitters, organic acids, phospholipids, sphingolipids, glycerides, and cholesterol esters, were identified and imaged to characterize their tissue-specific distribution in kidney tissues. Some metabolites, such as choline, acetycholine, betaine, phosphocholine, and glycerolphosphocholin were found to have distinct distribution along the cortex-medulla axis, which may be involved in the formation of osmotic pressure gradient in the kidney. The proposed ultra high resolution mass spectrometry based AFAI-MSI method can work without sample pretreatment, showing high sensitivity and wide metabolite coverage, and is expected to provide a new analytical approach in the research of *in situ* characterization and metabolic regulation mechanism of endogenous metabolites in kidney.

Key Words: Air flow assisted ionization; Mass spectrometric imaging; Endogenous metabolites; Kidney

1 Introduction

Mass spectrometry imaging (MSI) is a novel imaging technique that combines imaging processing software with mass spectrometry ion-scan techniques, and enables "direct" analysis of molecules in biological tissues at molecular level, providing information for their structures, contents, and spatial distributions[1,2]. Kidney is an important metabolic and excretive organ. Metabolic disturbance of endogenous molecules plays a crucial role in the pathogenesis of various kidney diseases such as diabetic nephropathy. Thus, it is important to get the whole picture of content and distribution of endogenous metabolites *in situ*, which may help reveal the pathogenesis of kidney diseases, and is conductive to the discovery of tissue-specific biomarkers[3–5]. At present, MSI is mainly based on three types of ionization techniques: secondary ion mass spectrometry (SIMS) and matrix assisted laser desorption ionization (MALDI) which both proceed ionization under vacuum condition, as well as ambient ionization technique developed in recent years and represented

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*Corresponding author. E-mail: zeper@muc.edu.cn
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by desorption electrospray ionization (DESI)[6–10]. SIMS is mainly used for analysis of elementals on the sample surface, while MALDI-MSI is the most developed and widely used MSI technique, especially for the imaging analysis of biological macromolecules such as proteins and peptides[6,7]. Recently, MALDI-MSI has also been applied in the field of endogenous small molecules imaging with the development of new matrixes. For example, Liu *et al*[11] investigated the pathogenesis of renal fibrosis in animal models by MALDI-MSI based on a novel salt-tolerant matrix, and found that the contents and distribution characteristics of 21 endogenous metabolites related to the metabolic networks of glycolysis, tricarboxylic acid cycle, fatty acid metabolism and antioxidants in animal models of renal fibrosis changed significantly. As compared with MALDI-MSI, DESI-MSI can work in the ambient environment without addition of matrix, and have advantages of easy-to-implement and no need of complicated sample pretreatment process[9,10], thus has a broad application prospect in the imaging analysis of small molecule metabolites. For example, Dill *et al*[12] compared the lipid metabolic profile of human papillary renal cell carcinoma with adjacent normal tissues, the results indicated that DESI-MSI combined with multivariate statistical analysis could distinguish carcinoma and normal tissues accurately, and was a promising technique for molecular pathological diagnosis of diseases. In our previous work, our group developed independently a new ambient air flow assisted ionization (AFAI; or air flow assisted desorption electrospray ionization, AFADESI), and its novel label-free, easy-to-implement, and highly sensitive mass spectrometry imaging (AFAI-MSI) technique, and applied it successfully in researches of mechanism of drug candidates, *in situ* screening of tumor biomarkers, and label-free molecular pathological diagnosis[13,14]. In this study, an AFAI-MSI method was established to investigate the tissue distribution of a variety of endogenous metabolites in rat kidney, and the results provided an important basis for the application of AFAI-MSI in the study of renal diseases.

2 Experimental

2.1 Instruments and reagents

AFAI-MSI system was constructed on a Q-Orbitrap mass spectrometer (Q Exactive, Thermo Scientific, USA) equipped with a custom-made AFAI ion source and Xcalibur 2.2 data acquisition and processing system. UltiMate 3000 Series Ultra High Performance Liquid Chromatography (Thermo Scientific, USA) included a binary gradient pump, an online degasser, an autosampler with a thermostat, a column oven, and a diode array detector.

All the tissue sections were prepared by a Leica CM1860 cryostat (Leica Microsystem, Germany), high-performance liquid chromatography (HPLC)-grade acetonitrile and isopropanol were purchased from Merck (Muskegon, MI). Ultrapure water (A particular brand, China) was obtained from a local market. Female Sprague-dawley rats (8 weeks age) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.

2.2 Tissue dissecting

Two female Sprague-dawley rats were asphyxiated to death with high concentration of CO_2, the kidneys were removed immediately and gently flushed with ultrapure water to remove the blood on the surface. After snap-frozen with liquid nitrogen, the tissues were stored at –80°C until use.

2.3 Tissue sectioning.

Two adjacent sections of kidney tissue with a thickness of 8 μm were obtained using CM1860 cryostat. The sections were thawed and mounted onto glass slides, and dried in a vacuum desiccator for about 30 min before AFAI-MSI analysis.

2.4 Mass spectrometry parameters

The mass spectra were acquired in positive mode, with the scan range of 70–1000 Da, mass resolution of 70000, the automatic gain control target of 3×10^6, and the maximum injection time of 200 ms. The spray voltage and transport tube voltage was set to 7 and 3 kV, respectively. Nitrogen (0.6 MPa) and isopropanol-acetonitrile-water (4:4:2, *V/V*, 5 μL min^{-1}) was used as spray gas and spray solvent, respectively. Data were acquired using Xcalibur software (Version 2.2, Thermo Scientific, USA).

2.5 Data processing

Raw data files were converted to the cdf format using Xcalibur software. Information on type, relative intensity and spatial position of the ions were read to perform imaging analysis using the custom-developed software MassImager (in corporation with Chemmind Technologies Co., Ltd)[15].

3 Results and discussion

3.1 Selection of solvents

The physichemical properties of spray solvents, such as polarity, viscosity, surface tension, dielectric constant and so on, may affect the extraction, desorption and ionization of target molecules from the tissue. Different proportions of methanol, acetonitrile, isopropanol and water were tested as the spray solvents, and the number and intensity of peaks were monitored to optimize composition and proportion of the