学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Application of Benchtop NMR for Metabolomics Study Using Feces of Mice with DSS-Induced Colitis
（卓上 NMR を用いた DSS 誘発大腸炎モデルマウスのメタボロミクス研究）

Metabolomics aims to comprehensively measure the metabolites, which are the downstream product of genes, transcripts, and protein functions. It has been applied to identify key biomarkers and investigate the pathogenesis of various human diseases. High-field NMR spectrometer based on the superconducting magnet has been one of the most routinely used techniques for metabolomics studies owing to its inherent advantages of being non-destructive, requiring a short analysis time and little sample preparation. However, further applications for medical purposes and field research are restricted and far from routine utilization because of its large size, substantial investment, and requirement of cryogenic fluids maintenance and well-trained staff.

The newly developed cryogen-free, low-field, benchtop NMR based on compact permanent magnets offer a potential solution to these challenges and may represent a new approach for metabolomics studies, benefiting from its small size and low running cost. In previous studies, the metabolic signature of type 2 diabetes has been profiled using urine samples. Moreover, tuberculosis in both humans and bovines was differentiated by benchtop NMR-based metabolomic fingerprinting using urine and plasma. Nevertheless, the feasibility of benchtop NMR for metabolomics studies has not been universally verified and the shortcomings of low sensitivity and resolution need to be solved. Therefore, to demonstrate the feasibility of benchtop NMR for fecal metabolomics, I employed the low-field NMR (60 MHz) to characterize the metabolic profile of feces samples from dextran sodium sulfate (DSS)-induced colitis mice, a commonly used model for inflammatory bowel diseases (IBD). I compared the results obtained from the benchtop NMR with those acquired using high-field superconducting NMR (800 MHz).

In this study, six male C57BL/6JJcl mice were divided into the control group and DSS-induced group and cultivated for 7 days. The same diet was given to all mice while 3.5% of DSS was added into the drinking water for DSS group mice to induce colitis. The feces sample were collected each

day for measurement by both 800 MHz and 60 MHz NMR spectrometers.

Forty-one metabolites were identified in a representative 800 MHz ^{1}H NMR spectra of concentrated fecal extracts from a healthy mouse. By referring to the assignment of 800 MHz spectra, nineteen metabolites were annotated to the 60 MHz spectra, including amino acids, short-chain fatty acids (SCFAs), creatine, formate, glucose, glycerol and lactate. To determine whether the metabolomics analysis based on 60 MHz NMR spectra performed effectively to discriminate the control group and DSS-induced group and provided comparable results with 800 MHz, multivariate analysis was performed for both 60 MHz and 800 MHz data. The analysis of 60 MHz data revealed that the metabolic signature of the DSS group began to separate from the control group from day 2 and completely separated from day 3, which showed the same trend with the 800 MHz data.

A higher intensity of 1.9 ppm derived from the acetate has been characterized as the most important feature in the NMR spectra acquired from the of DSS-induced mice. The high concentration of the acetate compared with the other metabolites and its singlet peak facilitated its easy detection. Thus, we expected to quantify the concentration of acetate as a key biomarker in our model to discriminate between the two groups and substantiate the potential of 60 MHz benchtop NMR for further quantification analysis. Three methods were attempted to quantify the acetate in the pure sample and feces sample: 1) simple integration; 2) “Generalized Lorentzian” (GL) curve fitting and 3) curve fitting using an in-house prepared database. Three methods showed good linearity and reproducibility for quantifying the pure sample. On the other hand, the simple integration method showed a large difference with the reference concentration based on the routine method using 800 MHz data. In contrast, no significant error was found between low-field and high-field data using the curve fitting methods, suggesting their high accuracy. Consistent with 800 MHz data, the quantification of the 60 MHz NMR spectra demonstrated a significantly higher concentration of acetate in the DSS group at day 5.

In summary, this study presented the potential applications of low-field benchtop NMR for the rapid diagnosis of IBD using a DSS-induced mouse model. The metabolic profile characterized by 60 MHz data showed good comparability with the 800 MHz data. In addition, the concentration of acetate, identified as a metabolite with characteristic behavior, could be accurately quantified using a “Generalized Lorentzian” curve fitting method based on the 60 MHz NMR spectra.