Analysis of Metabolites in the Urine of Mice with Tyrosinemia Type 1 using NMR and MS

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Background

- Hereditary Tyrosinemia Type 1 (HT1) is a genetic disorder resulting from a mutation in the fumarylacetoacetate hydratase (FAH) gene, producing a dysfunctional enzyme for the metabolism of tyrosine. This causes a build-up of tyrosine and other metabolites in the organs and tissues, harming the kidneys, liver, and brain. Currently the only drug on the market is Orfadin (NTBC).1
- A mouse model of HT1 was used to test social and cognitive function. Diseased mice taking NTBC were compared with healthy mice taking NTBC and diseased mice not taking the drug. Independently of NTBC treatment, mice with HT1 showed reduced learning and ability to adapt.1,3
- Eight key metabolites in the HT1 mouse urine have been identified. Current work is in developing a metabolomics protocol for analyzing the mouse urine components using NMR and LC-MS.
- The difficulty in this project arises from the complexity of the mixture. Metabolomics databases contain approximately 75,000 different metabolites and identifying individual components requires careful experimental design and execution.4 Beyond these metabolites are in an aqueous solution, which by itself is a challenge. Further complications arise from the inherently low concentrations of individual components.

Methods

- NMR experiments were run on a Unity INOVA® Varian 500MHz NMR, (H)PRESAT, PSYCHE, COSY, and HSQC experiments were used.
- An NMR stack of individual components of synthetic mouse urine is compared to a mixture in the figure below. Chemical interactions between components in the mixture result in different peak positions than for individual components. This helps in identifying peaks which are shifted in the urine sample due to similar shielding effects.
- LC-MS experiments were run on a Thermo Scientific Orbitrap XL®. LC-MS allows for the detection of components at low concentrations.

Figure 1: Stacked NMR spectra of individual metabolites compared with artificial mouse urine mixture.

Results

- A spectrum of artificial mouse urine was compared with a spectrum of mouse urine. Differences in shielding and complexity are notable.

Figure 3: NMR spectrum of mouse urine compared with artificial urine.

Figure 4: LC-MS Calibration curve with hydroxyindoleacetic acid response.

Conclusions

- A database of metabolites was drafted and a protocol for NMR and MS analysis of neurotransmitters and amino acids in mixtures developed. The NMR and MS detection limits for the eight key metabolites were also determined.
- Problems encountered included lack of sensitivity and overlap between peaks in the NMR spectrum. This was somewhat overcome by using multiple methods of analysis—NMR and LC-MS—and analyzing data with both VNMR® and MestreNova® software. 2D NMR experiments will be used in future work to minimize peak overlap.
- Further work includes building the database of metabolites, refining the metabolomic protocol, obtaining fresh mouse urine samples and analyzing them under standard conditions.

References


Acknowledgements

Dr. Cook and the RCEU staff for mentorship and guidance. The Alabama Space Grant Consortium, the Office of the Provost, the President and Vice President for Research, Dr. Christopher, Dean of the College of Science, and Dr. Wilkerson of the Honors College for funding. Dr. Gordon MacGregor for valuable input.

Figure 2: LC-MS plots used for calibration; concentration ranges 500 μg/L to 3.5 μg/L.