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Detection of Metabolic Changes caused by Antibiotics, Probiotics and High Protein Diets using Nuclear Magnetic Resonance Metabolomics

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INTRODUCTION

Many of the human body processes take place as a result of the numerous microbial communities residing within us. To better our understanding of the relationship occurring between host-resident microbes we must look further than the species and genes of the residents. Metabolomics is a technique that allows us to assess the functionality of how the host and microbes are integrated through examining biological fluids and tissues (Overmyer et al., 2015). These small molecules can show a distinct fingerprint of microbiota functionality and give insight to the health of a host. Confirming the identity of the single small molecules can present quite a challenge. However, when accomplished, the findings offer the ability to see into the chemical messengers that mediate interactions of the microbes amongst themselves as well as with the host they reside in. Nuclear Magnetic Resonance Spectroscopy (NMR) allows for the measuring of a wide range of small molecule metabolites within complex samples in a way that is reproducible (Klein, Oefner, & Gronwald, 2013). Through manipulating the microbiome composition via administration of antibiotics, perhaps we can observe the changes that occur in metabolism to better understand the health impacts of antibiotics.

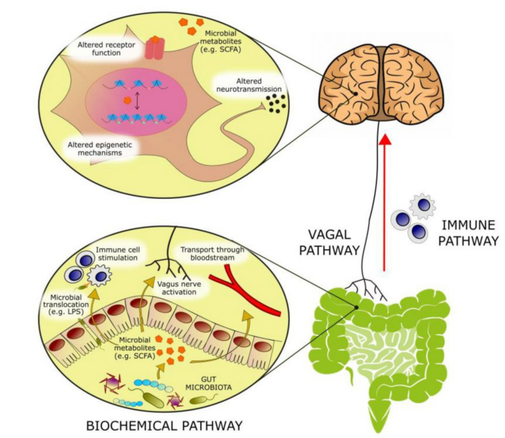

Figure 1. *Bioactive molecules originating from microbial metabolism are thought to modulate emotional behavior through several mechanisms (microbialcell.com)*

AIM

The overall objective of this study is to detect metabolic changes caused by antibiotics, high protein diets, and the New Generation Probiotics (Wang et al., patent pending) using Nuclear Magnetic Resonance (NMR) metabolomics.

METHODS

Animal handling

47 Male C57BL/6J 5-week-old adult mice were given either combination diets of standard and soy, standard and natto or standard diet alone, each at libitum. This part of the study was conducted by the Wang lab (Columbus, OH).

Table 1. *Mouse experimental treatment groups*

Experimental Group	probiotics	OG anitibiotics	TI antibiotics	ibuprofen	natto	soy	No. of animals
Control	no	no	no	no	no	no	12
New Generation Probiotics	yes	no	no	no	no	no	9
Antibiotic Tail Injection	no	no	yes	no	no	no	4
Ibuprofen	no	no	no	yes	no	no	4
Natto	no	no	no	no	yes	no	3
Soy	no	no	no	no	no	yes	3
Antibiotic by Oral Gavage	no	yes	no	no	no	no	6
OG Antibiotic and Natto	no	yes	no	no	yes	no	3
OG Antibiotic and Soy	no	yes	no	no	no	yes	3

Tissue collection

Euthanasia was performed by introduction of 100% of CO_2 where death occurred within 2.5 min., then tissue collection was initiated immediately. To prep serum, blood clotted at 25°C for 15 min. and was then centrifuged for 5 minutes at 5 °C, at 14,000 RCF. The supernatant was withdrawn and frozen at -80°C and stored until extraction.

Blood serum extraction and tissue extraction

Blood serum was mixed with the 4-fold amount of a 1:1 chloroform and methanol. Each sample was vortexed for 30 s, and incubated at -20°C for 60 min. The samples were then centrifuged at 14,000 RCF for 30 minutes at 4°C. For the tissue samples 100mg of tissue (liver or brain) were added to each of the bead (750 mg of beads) filled microtubes. 400 uL cold methanol (-20°C) and 85 uL of H_2O were added to each tube and homogenized for 30s in a Geno/Grinder homogenizer (Metuchen, NJ, USA) at 1400 rmp. The homogenates were transferred to microtubes. 400 uL of regular chloroform and 200 uL of H_2O were added to the homogenates and vortexed for 60 s. The microtubes were then iced for 10 min. at -80°C. The samples were then centrifuged for 5 min. at 1600 RCF at 4°C. For both the serum and tissue samples the upper, aqueous layer was transferred to a new microtube and evaporated. The dried samples were then dissolved in 550 uL of D_2O. 50 uL of D_2O containing 29.02 mM 3-trimethylsilyl-2,2,3,3- tetradeuteropropionate (TSP) as internal standard. The pH was adjusted manually to 7.4 with phosphoric acid dissolved in D_2O at 1% or Sodium Deuteroxide dissolved in D_2O at 1%. The adjusted serum samples were then transferred into Norell 5mm NMR tubes.

NMR spectroscopy

1D ^1H NOESY and ^1H-^{13}C HSQC experiments were performed on an 850 MHz Avance III HD spectrometer (Bruker, Rheinstetten, Germany) with a triple resonance cryogenic probe and z-gradients.

Data analysis

Principal Component Analysis (PCA) was completed on processed 2D NMR data using the AMIX 3.9.15 (Bruker BioSpin, Rheinstetten, Germany) statistical analysis tool to depict differences among metabolic profiles of mice after antibiotic and probiotic treatment. Spectral binning was done to minimize effects from differences in peak positions, and univariate analysis was performed based on experimental group type. Data was imported in R (The R Foundation, Vienna, Austria), noise signals were removed, and data was scaled using probabilistic quotient normalization. Linear models were calculated using the following factors: Probiotics, Oral Antibiotics, Injected Antibiotics, Ibuprofen, Natto, Soy, and combinations of oral antibiotics and soy or oral antibiotics and natto. p-values were adjusted using False Discovery Rate Controlling.

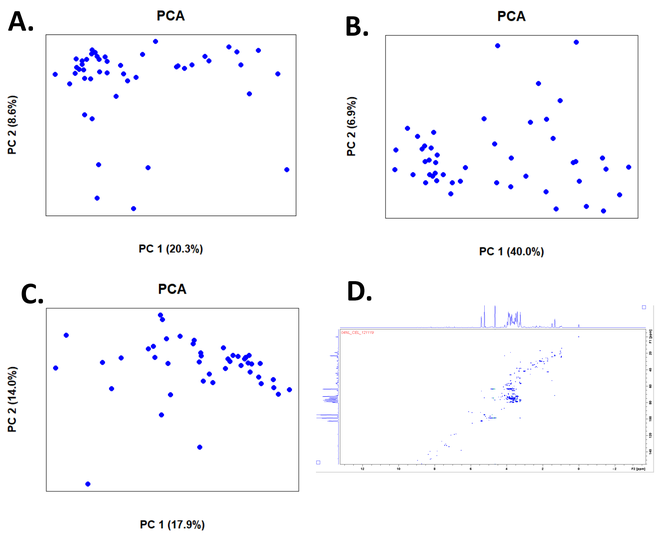

Figure 3. *Principal Component Analysis for 2D NMR data (**3D** is an example of a 2D NMR Liver Spectra) PCA of the **3A** Brain, **3B** Liver, and **3C** Serum metabolite data.*

RESULTS

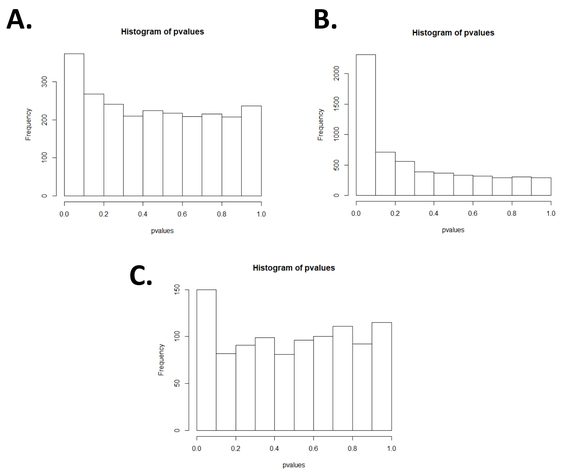

Figure 2. *A histogram display of the pvalues distributed among **2A**. Brain **2B**. Liver and **2C**. Serum samples.*

Results show strong metabolic differences between diets and after antibiotic or probiotics administration both in the tissue samples and the serum. The pvalues of both tissue samples and serum show similar histogram displays with the most frequency below .05 (Figure 2).

CONCLUSIONS

Differences in diet were able to be distinguished metabolically according to metabolite data.

Further analysis is needed to identify the molecules changed according to diet and probiotic treatment. As we discover more about the phenomena occurring within the gut after antibiotic administration and factors contributing to a loss of homeostasis, we can more accurately define the mechanisms of interaction between the host, commensal microbes and pathogens to make better decisions for our health when it comes to standards of practice, and choice of diet.

BIBLIOGRAPHY

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