Gut Microbial Metabolites and Hepatic Xenobiotic Metabolism: A High Throughput Screening Approach

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Introduction

This poster depicts the development of a novel exploratory pipeline to investigate the impact of gut microbial metabolites on xenobiotic metabolism.

The gut microbiota are the vast population of microbes residing in the gut. Collectively, these microbes possess a diverse array of biochemical functions that are not encoded in the mammalian host genome. As such, the microbiota produce unique metabolites that can interact with host metabolic processes and influence the overall metabolic status of the host. Understanding the interaction between the gut microbiota, their metabolites and hepatic metabolism may provide novel insights into idiosyncratic drug responses. Idiosyncratic drug responses occur in 15% of treated patients, where a normal therapeutic dose becomes toxic. These idiosyncratic effects cannot be reproduced in animal models making them difficult to model and study. **We hypothesise that inter-individual variation in the gut microbiome may influence host drug metabolism and toxicity through production of microbial metabolites.** Here, metabolic profiling was used to identify metabolic variation in the urine of rodents treated with antibiotics. The potential of these microbial-associated metabolites to impact on host drug metabolism was explored using a high throughput hepatic *in vitro* cell model designed to focus on cytochrome P450 CYP2E1, a key phase I drug metabolising enzyme. CYP metabolism produces toxic intermediates which are likely to be responsible for adverse drug responses.

Aim

- **Identify microbial metabolites by comparing the urinary metabolic profiles of conventional and antibiotic-treated rodents.**
- **Develop a high throughput hepatic screening model for identifying the toxicity of gut microbial metabolites via the CYP2E1 pathway.**
- **Establish whether gut microbial metabolites can exert an idiosyncratic response when co–dosed with paracetamol (APAP).**

Antibiotic-treated rat study

- Male Wistar rats (*n* = *6*) were provided with penicillin and streptomycin in their drinking water for 8 days. A control group (*n* = 6) received antibiotic-free drinking water. Urine was collected at day 8 and the metabolic profile was measured using ^{1}H nuclear magnetic resonance (NMR) spectroscopy.

In vitro screening model methodology

- THLE cells were selected as 96 ADME genes have be characterised.
- THLE cells lack SULTs and UGTs involved in phase II drug metabolism allowing the phase I metabolism of microbial metabolites to be studied.
- This model was developed to focus on the CYP2E1 metabolism of microbial metabolites identified from the in vivo antibiotic study.
- Easy to maintain and reproducible data up to passage 30, used at 4,000 cells per well (384 well plate) ideal for a high throughput screen.
- Glutathione depletion was explored by pre-treating cells with L-Buthionine sulfoximine (BSO) for four hours followed by dosing with microbial metabolites (24 hours).
- Luciferase was used to determine ATP concentration, an indicator of cellular health and luminescence was read by a PerkinElmer EnVision.

Results

Attenuation of the gut microbiome through antibiotic administration modulated the excretion of several microbial-associated metabolites

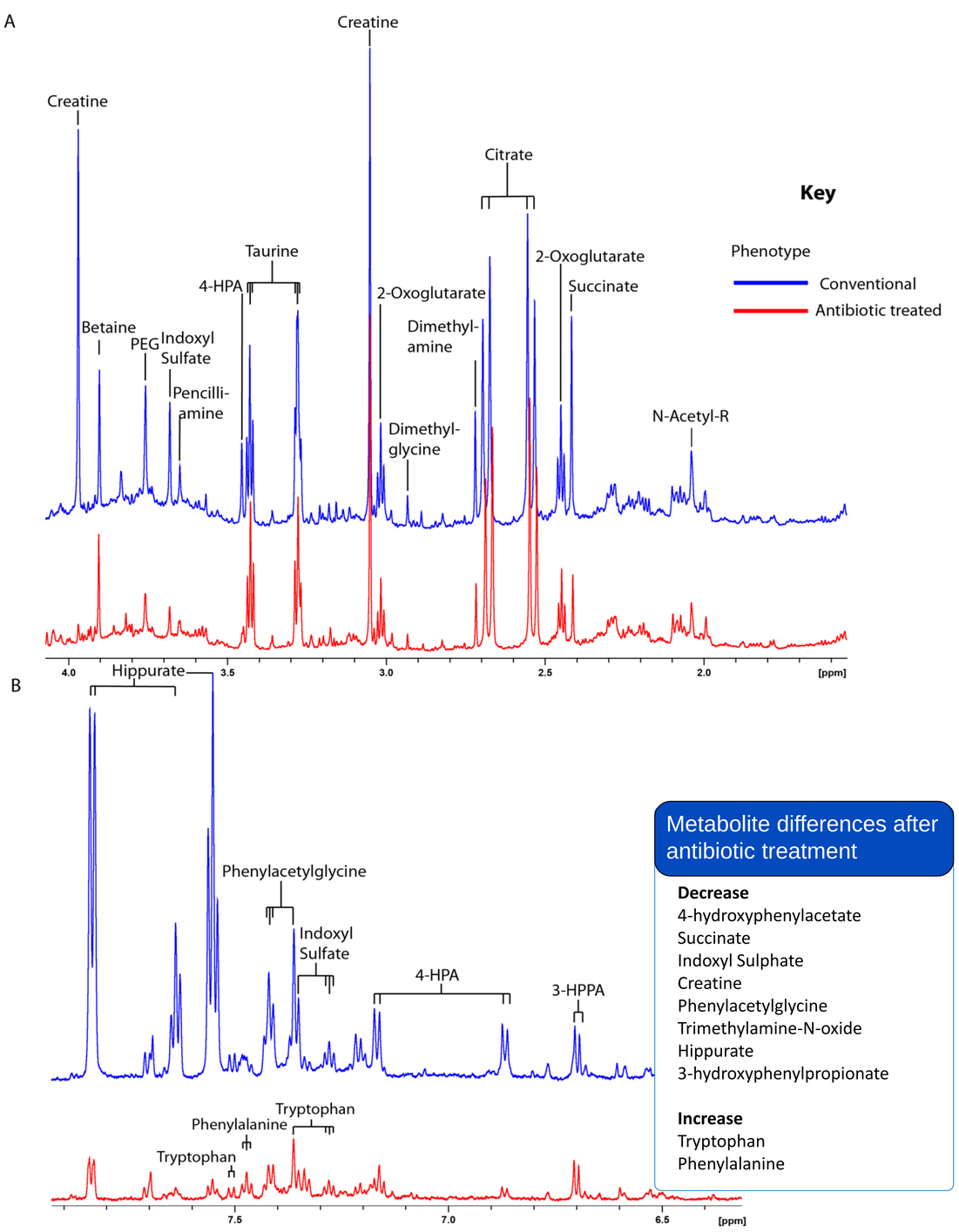

Figure 1: ^{1}H NMR spectra highlighting the differences in urinary metabolites between antibiotic-suppressed and conventional rats in (A) aliphatic and (B) aromatic regions.

THLE screening revealed that CYP2E1, a phase I enzyme, increases the toxicity of 4-cresyl

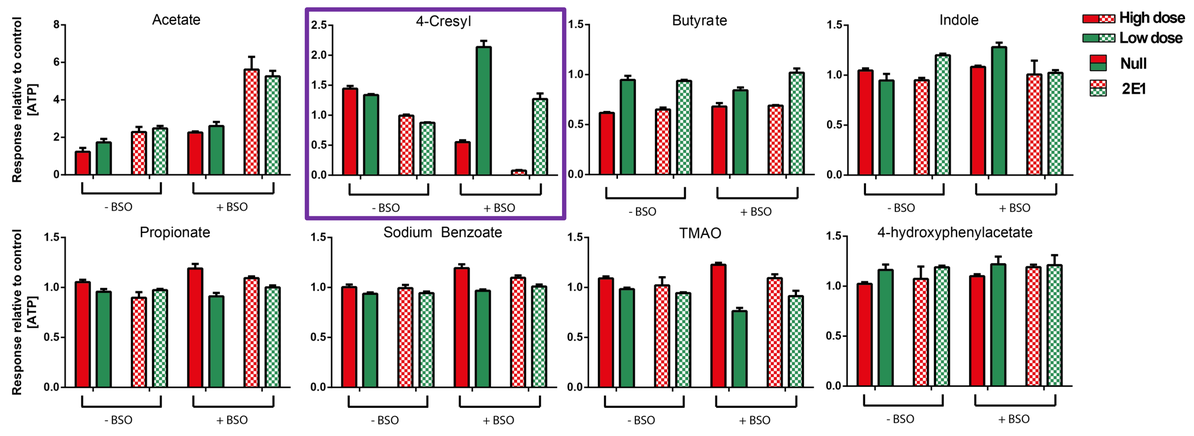

Figure 2: Graphical representation of cytotoxicity (ATP concentration) of the highest and lowest doses of eight microbial-associated metabolites in THLE Null and THLE CYP2E1 cells with or without glutathione depletion (BSO)

Co-dosing 4-cresyl and APAP enhances the toxicity of APAP in CYP2E1 cells

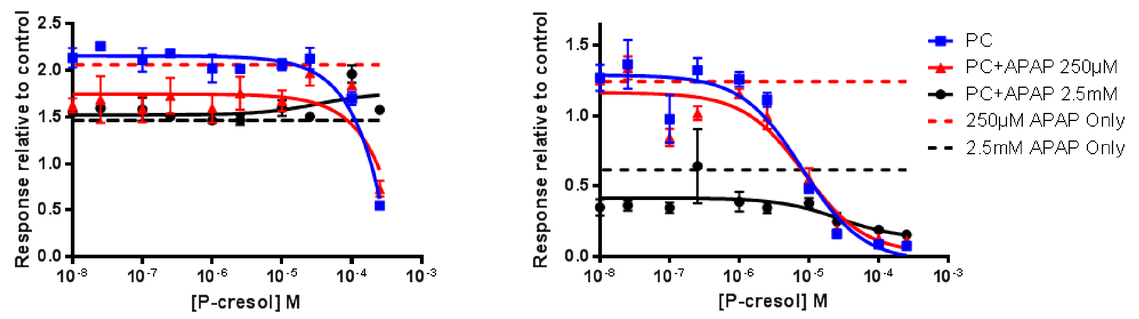

Figure 3: Co-administration of PC and APAP had minimal effects on cytotoxicity in THLE Null cells. In THLE cells transfected with CYP2E1, the co-administration of *p*-cresol was found to increase the cytotoxicity of APAP at 2.5 mM. This followed a dose-dependent response. This effect is mediated by CYP2E1converting APAP and PC to their toxic intermediates.

Conclusion

- From the urinary metabolic profiles, 12 microbial-associated metabolites were found altered following antibiotic administration.
- 4-cresyl was found to be cytotoxic after CYP2E1 metabolism and this toxicity was increased with GSH depletion.
- In CYP2E1 cells, 2.5 mM APAP toxicity was enhanced when co-administered with 4-cresyl (10nM to 1µM).
- **Combining metabonomics and high throughput THLE cell screening provides an effective pipeline to identify gut microbial metabolites and determine their potential role in drug induced liver injury caused by idiosyncratic drug responses.**

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