MUNI | RECETOX

Automated functional metabolomics and application to case-control disease studies

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INTRODUCTION

Significant interaction between chemical exposure agents, dietary and microbiome-derived chemicals has been recorded[1], affecting disease progression and treatments. However, the interplay between these factors is not well understood. Methods and resources based on mass spectrometry aimed for enhancing understanding between these factors will be developed. The experiment will consist of two main parts:

1) Short chain fatty acids (SFCAs) assay

- SCFAs are water-soluble fatty acids of 2-6 carbons (Figure 1)
- Measures of SCFAs are indicative of host gut-microbiome crosstalk[2]
  - Energy source for gut epithelium, modulate intestinal integrity
  - Products of fiber digestion evidencing gut microbiota functionality
  - Provide prevention against pathogen gut colonization

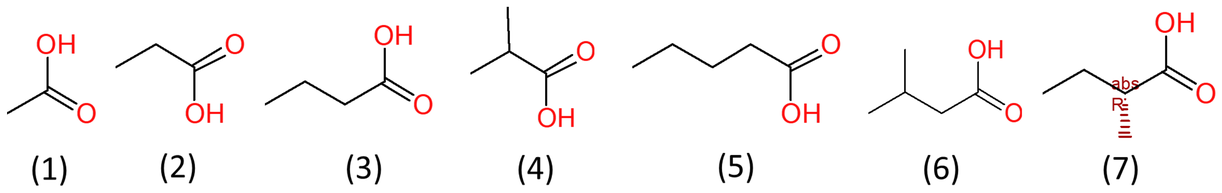

Figure 1. *SCFAs structures showing Acetic acid (1), propionic acid (2), butyric acid (3), isobutyric acid (4), valeric acid (5), isovaleric acid (6), 2R-methylbutyric acid (7).*

Method development

- Basis is short (12.5 minute), simple (solvent extraction) assay[3]
  - 100 µL serum or plasma, extracted with acidified methyl tert-butyl ether
  - Gas chromatography–(selected ion monitoring) mass spectrometry; GC-[SIM]-MS
  - Quantitation via stable isotopic labelled standards

- Translate GC-[SIM]-MS to GC-[full scan/SIM]-MS (Figure 2)
  - Enables combined screening and quantification
- Automate sample preparation
  - Enhance reproducibility and throughput
- Adapt for fecal swab samples
  - Greater potential for neonatal studies

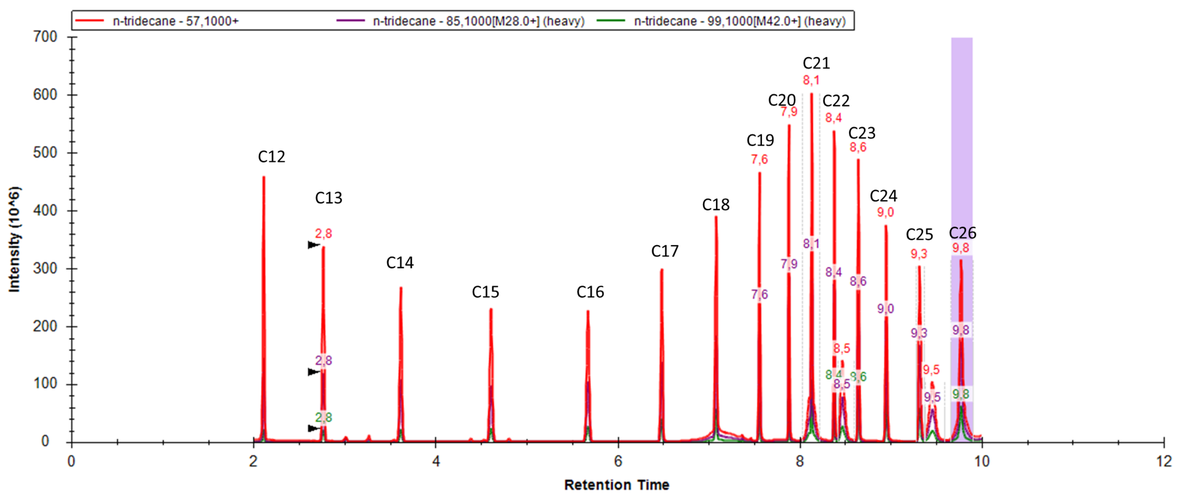

Figure 2. *Example chromatogram of translated GC method showing C12-C26 alkane separations (50 ng on column). SCFAs elute between C14 – C17.*

Future

- Established method will be transferred to routine operations within the Biomarker Analytical Laboratories Research Infrastructure (BAL RI)
  - SOPS to be developed and made public

- Method to be applied to measure samples from cancer (testicular & hematologic) survivor cohorts
  - Cooperation with Comenius University, the National Cancer Institute & the Biomedical Research Center of the Slovak Academy of Sciences

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2) Synthesis of biotransformation products of drugs

- The liver in the primary site of biochemical modification of internalized chemical agents
  - Highest activity of enzyme family cytochrome P450 (CYPs)

- The detoxification process is classically described in 3 phases:
  - **Phase I:** modification introducing reactive groups e.g., oxidation via enzymatical group of cytochrome P450s (CYP), reduction, hydrolysis etc.
  - **Phase II:** conjugation to larger molecules, typically with reduced bioactivity and more polar e.g., glucoronidation, sulfation, methylation etc.
  - **Phase III:** additional modification of phase II analytes
- At all three stages, analytes are relocated to kidneys or gallbladder for excretion via passive and active transport mechanisms

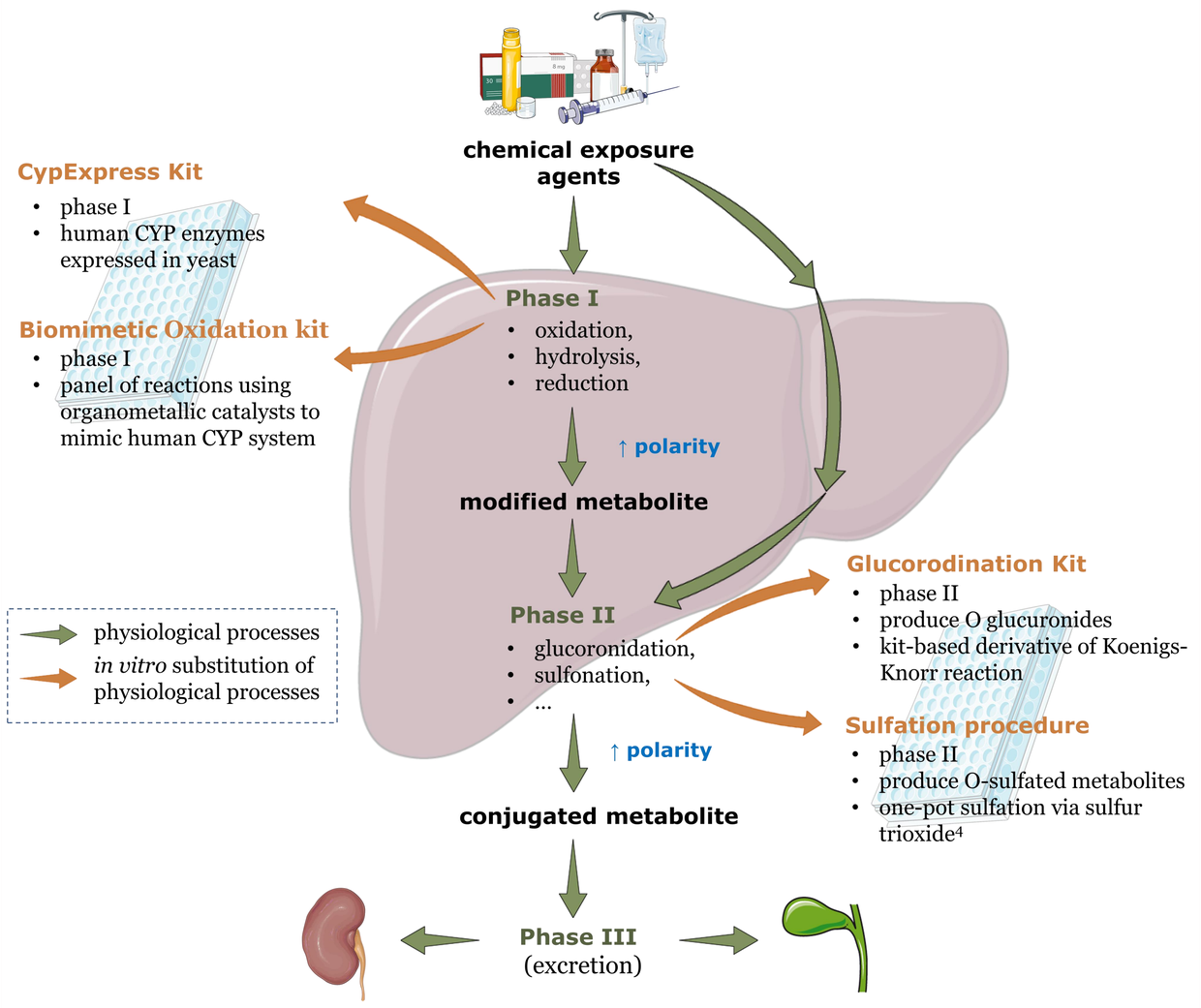

Figure 3. *Biotransformation in human body and substitutions for producing drug metabolites in vitro.*

Proposed work

- Producing biotransformation products *in vitro* using one-pot chemical synthesis, biomimetic reaction kits and recombinant yeast expression systems (Figure 3)
  - Parent drug molecules from Prestwick library containing ~200 most prescribed drugs

- Characterizing the metabolic products using GC-HRMS or LC-HRMS
  - Specific emphasis on sulfated analytes indicative of phase II drug detoxification and/or gut microbiome co-metabolism

Future

- Open high-resolution mass spectral library resource of biotransformation phase II and phase III products

- Retrospective mining of previously acquired and public datasets to enhance annotation rates, with emphasis on cancer treatment studies

- Combined application with SCFA assay to demonstrate value of developed resources to investigate host-microbiome and drug-nutrient interactions

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