Measuring Liver Function and Activity of Metabolic Enzymes with Breath Biopsy® and EVOC® Probes

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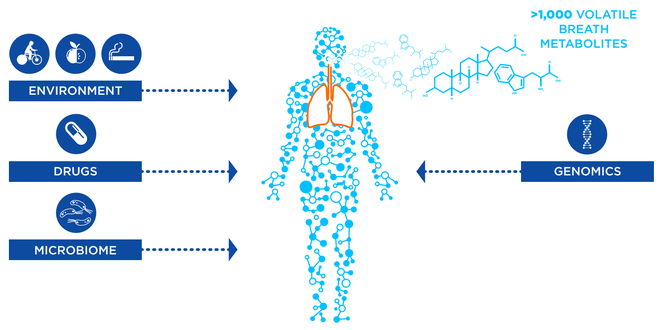
Introduction

- Hypothesis-driven administration of exogenous compounds could provide a means to investigate the analysis of selected compounds in breath
- We propose a **novel approach** that uses **Exogenous Volatile Organic Compounds (EVOC®) Probes** to measure the **activity of metabolic enzymes *in vivo***, as well as the **function of organs**, through **breath analysis**
- Pharmacogenomic approaches can determine metabolizer genotype, whereas **Breath Biopsy** can be used to determine a subject's **metabolizer phenotype**

What is Breath Biopsy and What are VOCs in Breath?

- Breath Biopsy is a non-invasive way to collect and analyze the chemical makeup of breath
- Volatile Organic Compounds (VOCs) are low molecular weight metabolites produced throughout the body. They are picked up and distributed in the bloodstream, and excreted via breath due to the rapid exchange of metabolites between the blood and breath in the lungs. VOCs can be of endogenous and/or exogenous origin
- Breath Biopsy enables researchers to explore the potential application of EVOC® Probes as tracers of specific *in vivo* metabolic activities, in order to determine a subject's metabolizer phenotype



CYP450 Liver Enzymes and Using Breath Biopsy to Determine Metabolizer Phenotype

- Cytochromes P450 (CYPs) are a family of enzymes involved in the elimination of foreign compounds (xenobiotics) such as drugs and toxins from the body
- The functioning capacity of a patient's CYP450 enzymes has a large impact on the efficacy of their treatment
- Ultra-rapid/rapid metabolizers and poor metabolizers require different doses to maximize their therapeutic window
- Pharmacogenomic approaches can determine the metabolizer genotype, but genotype is not a complete measure of how a patient would actually metabolize a drug. The metabolizer phenotype can be influenced by a number of other factors including diet, smoking, and other drugs the patient may be taking

Bioactive drug → **P450** → **Inactive metabolite**

70%

of drugs are metabolized by only four enzymes

Normal metabolizers change the bioactive drug into an inactive metabolite over the period of effect of the drug

Ultrarapid metabolizers may process the drug before it can be effective

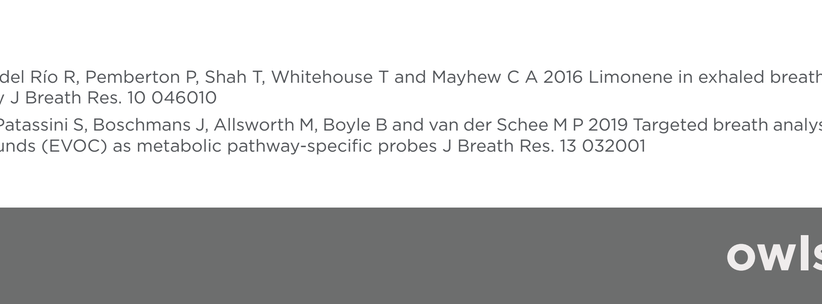
Poor metabolizers may process the drug too slowly, leading to system buildup and **toxicity**

EVOC® Probes, comprised of compounds generally recognized as safe, are metabolized by the same CYP450 enzymes as drugs. Examples of EVOC® Probes are terpenes such as limonene and eucalyptol. By measuring the kinetics of a mix of EVOC® Probe substrates and metabolites on breath, we can investigate metaboliser phenotype.

Example EVOC® Probe	CYP450
Limonene	2C9,2C19
Eucalyptol	3A4
Linalool	2C19,2D6

Targeted Breath Analysis Using EVOC® Probes

- EVOC® Probes are volatile compounds that, administered to a subject through various routes, undergo metabolism and distribution in the body and are excreted via breath
- By examining the underlying biology and associated enzymatic pathways, EVOC® Probes can be identified that would interact with those pathways in a meaningful and dose dependent way
- The kinetics of metabolism and subsequent breath excretion of the EVOC® Probe, or of its products, could then be used as a readout of the metabolic activity of specific enzymes, such as the CYP450 enzymes
- Virtually any exogenous VOC that is metabolized by the human body can offer a readout of metabolic enzymes/organs



EVOC® Probes for *In Vivo* Metabolic Phenotyping

The kinetics of metabolism and subsequent breath excretion of the EVOC® Probe, or of its products, could enable in vivo assessment of the activity of specific metabolic enzymes and organ function.

- Breath clearance of the EVOC® Probe can be monitored in breath, as well as multiple products that can derive from metabolism of the EVOC® Probe by specific enzymes
- Can administer a cocktail of probes – test multiple targets
- Completely **non-invasive**
- Targeted hypothesis-driven approach

Ingestion of a capsule cause a measurable increase in the levels of capsule-related compounds in breath and a washout curve over time

References

O'Hara M E, Fernández del Río R, Pemberton P, Shah T, Whitehouse T and Mayhew C A 2016 Limonene in exhaled breath is elevated in hepatic encephalopathy J Breath Res. 10 046010

Gaude E, Nakhleh M K, Patassini S, Boschmans J, Allsworth M, Boyle B and van der Schee M P 2019 Targeted breath analysis: exogenous volatile organic compounds (EVOC) as metabolic pathway-specific probes J Breath Res. 13 032001

Terpenes as EVOC® Probes

- Ingestion of an EVOC® Probe resulted in a marked increase in concentration of terpenes in breath after 30 minutes, compared to baseline levels (Figure 1)
- As the terpenes are metabolized and gradually cleared, blood concentrations decrease over time, with progressively lower excretion in breath
- The washout kinetics that we observed suggest involvement of first-pass intestinal and hepatic metabolism

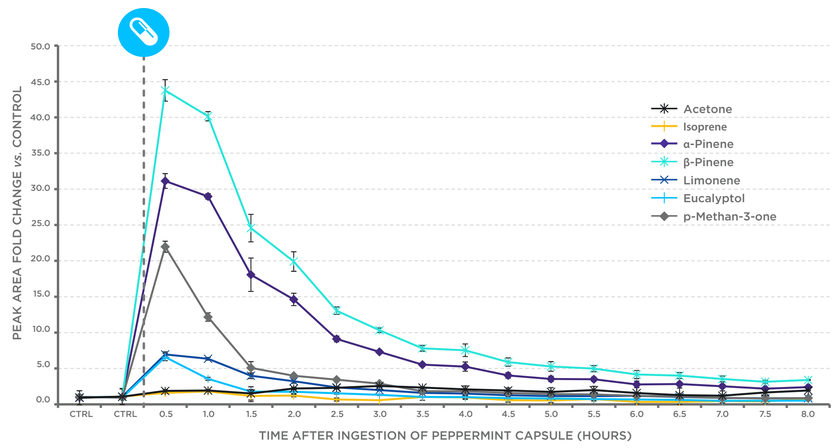
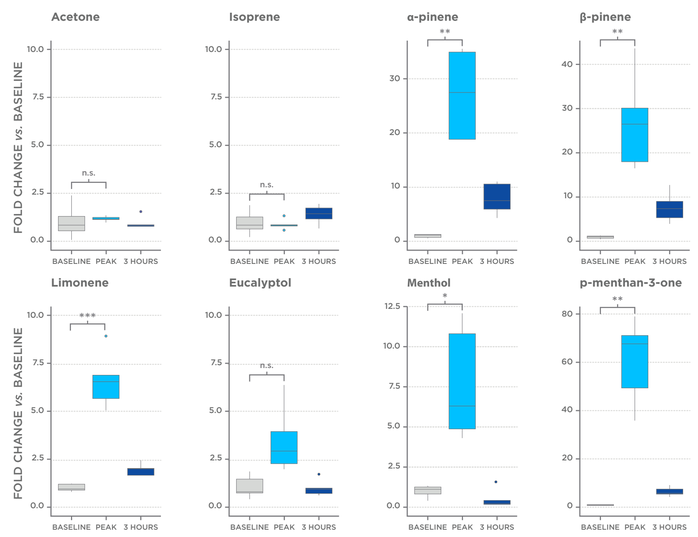


Figure 1: Washout curves of acetone, isoprene, and different terpenes/terpenoids from one healthy subject at baseline (Ctrl) and after ingestion of the EVOC® Probe (peppermint oil capsule). Breath samples were collected using the ReCIVA Breath Sampler and analyzed using the Breath Biopsy platform in the Breath Biopsy Laboratory (Owlstone Medical Ltd, UK).

*Breath samples from repeated collects from one individual over 8 hours (16 timepoints) were analyzed. Four replicate VOC sample tubes were collected and analyzed at each time point.

High Reproducibility in Breath Measurement of EVOC® Probes

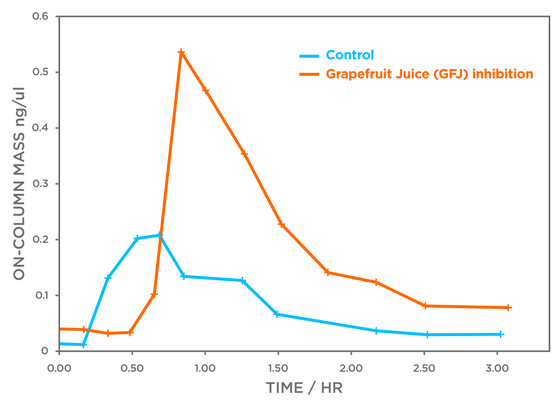


From the washout experiment, three time points were selected (pre-ingestion control, peak at 45 minutes and plateau at 3 hours) for inclusion in a longitudinal study where the washout experiment was repeated multiple times over 5 weeks (Figure 2).

Breath metabolites acetone and isoprene show only small differences between peak and plateau fold change as expected, however large fold changes are observed for terpenes such as α-pinene, β-pinene.

*Figure 2: Boxplots of breath levels of acetone, isoprene and different terpenes at baseline (before EVOC®), peak (45 min after EVOC®) and 3 hours after EVOC®, acquired across 5 weeks in one healthy subject. *, **, and *** represent paired t-student p-value < 0.05, 0.01, 0.001, respectively. n.s. = not significant.*

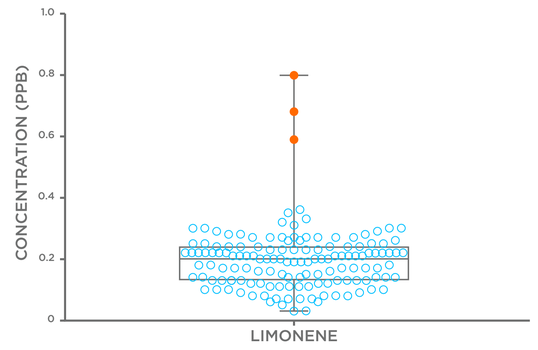
Inhibition of CYP450 Enzymes Alters The Kinetics of EVOC® Probe Metabolism



Administering the EVOC® Probe eucalyptol before or after ingestion of grapefruit juice (which inhibits CYP3A4) lead to altered washout kinetics, with increased levels of eucalyptol in breath following ingestion of grapefruit juice.

Figure 3: Eucalyptol washout curves before and after CYP3A4 inhibition by grapefruit juice. As CYP3A4 is inhibited, less of the substrate Eucalyptol is metabolized and so is seen at higher levels in breath.

Limonene Levels in Subjects with and without EVOC® Probe Administration



EVOC® Probes resulted in a significant increase in limonene levels, generating a separated distribution of breath limonene concentrations (Figure 4, orange circles), compared to background levels of limonene in 136 individuals (blue circles).

Figure 4: Comparison of background levels of limonene and EVOC-induced limonene changes. Breath concentrations of limonene were measured in 136 subjects (blue circles) and compared with limonene levels after EVOC® probe administration (orange dots).

Designing EVOC® Probes

- Our approach to EVOC® Probe development is one based on the underlying biology, understanding disease pathology and taking a close look at enzymatic pathways that underlie it
- We then work to identify potential probes that would interact with those pathways and validate them by confirming route of administration, distribution kinetics within the body, intra- and inter-individual variability, likelihood of secretion in breath, and finally determining dosage

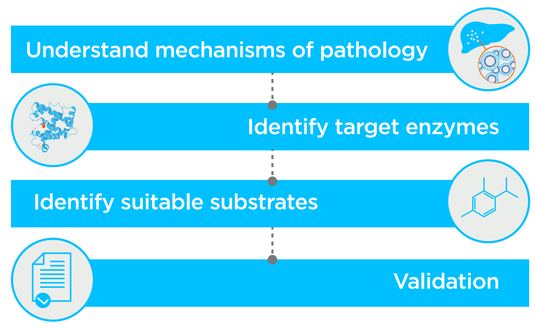


Figure 5: The development pathway of EVOC® Probes

Conclusions

- Owlstone Medical is exploring the development of a Breath Biopsy test using **EVOC® Probes** that are **metabolized by the same CYP450 enzymes that process medications**
- By administering one or more EVOC® Probes to a subject, and measuring the breath VOC levels and rate of processing and breakdown of the EVOC® Probe, we can potentially build a picture of the ADME profile of a subject relevant to a specific enzymatic pathway
- Using Breath Biopsy EVOC® Probes to measure an individual's metabolizer phenotype *in vivo* has the potential to be **more predictive than genotype alone**