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.
- Pharmacokinetic approaches can determine metabolizer genotypes, 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 potentially exploit EVOC® Probes as traces of specific in vivo metabolic activities, in order to determine a subject’s metabolizer phenotype.

Terpenes as EVOC® Probes

- Ingestion of an EVOC® Probe resulted in a marked increase in concentration of terpenes in breath after 30 minutes, which are elevated in poor metabolizers (Figure 2).
- 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.

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 kinetic 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.
- 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 metabolizers and poor metabolizers require different dosages to maximize their therapeutic window.
- Pharmacokinetic approaches can determine the metabolic genotype, but genotypes is not a complete measure of how a patient will metabolize a drug.
- In vivo and in vitro results are often confounded by a number of other factors including diet, smoking, and other drugs the patient may be taking.

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Metabolizer Phenotype

- In a healthy subject, 70% of drugs are metabolized by the CYP450 enzymes.
- In Healthy subjects, the majority of drugs are metabolized by the CYP450 enzymes.
- Poor metabolizers may process the drug before it can be effective, changing the bioactive drug into an inactive metabolite.
- Normal metabolizers can derive from metabolism of the EVOC® Probe by specific enzymes.

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).
- Breathing metabolites acetone and isoprene show small differences between peak and baseline levels (Figure 3).
- However, large fold changes are observed for terpenes such as limonene, pinene.

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

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

Limonene Levels in Subjects with and without EVOC® Probe Administration

- Figure 4: Comparison of background levels of limonene and EVOC-induced limonene changes. Breath concentrations of limonene were measured in 135 subjects (blue circles) and compared with baseline 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 these pathways and validate them by confirming route of administration distribution kinetics within the body, intra- and inter-individual variability, half-life of secretion in breath, and finally determining dosage.

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.

References


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