Non-invasive tools that are effective for disease screening, diagnosis and monitoring remain an unmet need in many disease areas. This article discusses breath volatile organic compounds (VOCs) as non-invasive biomarkers, and how breath, in addition to faecal VOCs, has the potential to reflect gut microbial activities, making it highly favourable for clinical application. More importantly, the article highlights an important interaction between gut microbial-produced VOCs and drug action, and by taking this into consideration during drug development, the pharmaceutical industry could improve the efficacy and patient-targeting capabilities of new drugs.

Volatile organic compounds (VOCs) are a large group of carbon-based chemicals that are emitted from biological samples, such as faeces, urine and breath. They include molecules such as short-chain fatty acids (SCFAs) and alcohols, which are well known products of the microbiome. VOCs produced in the body come from cellular metabolic activities, reflecting the physiological state, and have high potential to serve as disease biomarkers. The collection of VOCs from all biological samples is non-invasive, however the measurement of VOCs on breath is preferable over other biological samples for several reasons including: ease of sampling and repeat sampling within a short time frame, samples taken in real-time reflecting metabolic activity at a precise time point, and reduced variability due to standardised sample collection.

Breath VOCs can originate from either within the body (endogenous VOCs) or from external sources including dietary and environmental exposures (exogenous VOCs). Changes to the levels of endogenous VOCs can be characteristic of specific disease processes as they are often products of metabolic activity in the body. However, many metabolites produced by the human body are not derived from human metabolism – they can also originate from microbes within the gut. VOCs produced by microbes in the gut have been studied far less despite these VOCs showing links to several disease areas including gastrointestinal diseases. Many of the gut microbial-produced VOCs come from the metabolism of dietary fibre. Dietary fibres, which cannot be metabolised by the human body, are fermented by gut microbes, the metabolism of which produces volatile SCFAs and alcohols. Other known volatile microbial metabolites include phenol, phenyl acetaldehyde and p-cresol, which are produced through aromatic acid catabolism. Many of these gut microbial-produced VOCs are beneficial to the body and may regulate both local and distant immune, neuronal and metabolic responses. Changes in the levels of these VOCs could therefore imply gut microbiota composition and/or diversity changes, as well as its associated diseases such as cancer, gastrointestinal disorders and cardiovascular disease.

Measuring Gut Microbial-produced VOCs in Breath

Faecal headspace analysis, which is the collection and analysis of components present in the volume of gas above a sample, has been most widely used to understand the role of the gut microbiome in digestive health. However, data from faecal headspace analysis does not reflect real-time metabolic activity and can be delayed by up to 48 hours. Also, variability is introduced due to the quick evaporation of VOCs from headspace samples, and the lack of standardised research protocols makes comparisons between studies difficult. Breath VOCs represent an alternative option as it is reflective of the same gut microbial-produced VOCs found in faeces, with all the aforementioned advantages of sampling and analysis. Taken together, breath analysis has emerged as a highly effective method for both biomarker research and clinical tests.

It is worth noting that whilst breath offers several advantages over faecal sampling, breath VOCs originate from all around the body and are not restricted to the gut. Headspace analysis of faecal samples is therefore a useful tool to first identify VOCs known to be produced in the gut, which can then be used to inform VOC targets in breath sampling. Comparison of the levels in breath and faecal samples can be then undertaken to ensure breath analysis provides an accurate representation of gut behaviour.

Gut Microbial-produced Short Chain Fatty Acids in Patients with Crohn’s Disease

The connection between exhaled breath VOCs and gut microbiota within the same subjects has been demonstrated in Crohn’s disease (CD), a subtype of inflammatory bowel disease (IBD). Through breath sample and faecal metagenomics analysis in both active and inactive CD subjects, SCFAs acetate and propionate significantly correlated with Bifidobacteria and several other microbes in the Firmicutes phylum in both disease states. More importantly, the relative abundances of produced SCFAs and the correlated microbial strains both decreased in active disease subjects, indicating how disease state, the gut microbiota and its produced VOCs are connected. Other findings from the study included significant correlations between inflammation VOC biomarkers like pentane and octane, and Bacteroides fragilis and Ruminococcus gnavus in active disease state subjects. While this does not imply the specific VOCs were produced by these bacteria, their level of abundance could share a common cause.

Gut Microbial-produced Ethanol in Non-alcoholic Fatty Liver Disease Patients

Gut microbes can metabolise a variety of diet-derived substrates through microbial fermentation. In liver disease, non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are thought to share a common pathophysiology with alcoholic fatty liver disease (AFLD). Rather than from alcohol intake, it is hypothesised that ethanol exposure in these diseases is due to gut bacteria overproducing ethanol from pyruvate, the end-product of glycolysis. However, peripheral blood concentrations of microbial-derived ethanol generally
were deemed negligible compared to the amount of ethanol that can be ingested.

Recent clinical studies have linked the development of NAFLD and NASH to microbial dysbiosis. Metagenomic analysis correlated specific bacterial populations with high levels of gut ethanol fermentation in subjects with NAFLD and NASH. However, the ethanol produced in the gut is metabolised by hepatic alcohol dehydrogenase before it reaches peripheral circulation, and therefore only a slight elevation of circulating blood alcohol was measured in these individuals when compared to healthy subjects.

Ethanol levels in the portal vein leading from the gut to the liver are elevated by up to 187 times with this increase even more pronounced in NAFLD and NASH individuals. Ethanol levels were determined using a selective inhibitor 4-methylpyrazole (4-MP) to inhibit hepatic alcohol dehydrogenase activity (Figure 1), and results showed that microbial-produced ethanol levels in the periphery strongly increased and spiked two-hours after meal consumption in these subjects relative to healthy subjects without steatosis.

When these subjects with NASH were given a broad-spectrum of antibiotics (metronidazole, clindamycin, ciprofloxacin) for one week to deplete the gut microbiome and ablate the ability for ethanol production after a meal containing carbohydrates, antibiotic treatment nearly completely suppressed peripheral ethanol levels in subjects given the alcohol dehydrogenase inhibitor and a carbohydrate meal (Figure 1). These findings support the idea of the gut microbiome driving overt ethanol production in individuals with NASH. There is therefore a need for a device capable of monitoring microbial-produced ethanol routinely and in a non-invasive manner.

The Importance of Taking Gut Microbial Activity Into Account During Drug Development

It is estimated that subjects with NASH can produce between 7 and 100g of ethanol per day, which is above the guidelines of alcohol intake safety concerns for subjects with NASH. It is concerning that a substantial proportion of NASH subjects should be excluded from the NAFLD category as they are exposed to endogenously produced ethanol, regardless of meeting the inclusion criteria for ethanol intake. This is important especially for the pharmaceutical industry, as large database analysis on prescription medications suggest potential alcohol interaction in 45% of the medications.

There are at least eight classes of new drugs under development for NASH treatment, each acting against different pharmacological targets aimed to alleviate or prevent further disease progression (Figure 2). Many of these drugs target metabolic processes that are exacerbated by chronic ethanol exposure, and therefore ethanol intake is strictly controlled. However, the lack of consideration for gut ethanol production may lead to undesired outcomes during drug development. Not controlling for ethanol produced by gut microbials could contribute to failure during clinical trials or ineffective identification of patient populations that most effectively respond to a given drug, reducing the likelihood of establishing the efficacy of a NASH drug.
While some NAFLD subjects are non-ethanol producers, it has been reported that up to 60% of NAFLD subjects have gut bacteria that produce up to 100g of ethanol per day. Given that the beneficial effects of NASH drugs can be counteracted by ethanol, it therefore follows that subjects with ethanol produced in their body would likely have reduced or absent efficacy towards drug response. Additionally, the detrimental effect of ethanol on drug efficacy may lead to a requirement for greater dosages for desired response, causing more side effects and safety concerns. To help identify patients that are more likely to respond to therapy in clinical trials, and to increase the potential of bringing new NASH drugs to market, a diagnostic test for gut ethanol production is an urgent need.

Taking into account the liver’s ability to metabolise ethanol from the portal vein and mask levels in the periphery, blood-breath partition ratios, etc, breath levels of ethanol from gut fermentation are expected to be less than 20 ppb on a volume basis. This means that currently available breath analysers are not sensitive enough, as they can only detect ethanol at levels greater than 100 ppb. Lab-based analytical instruments have the capacity to detect ethanol in the ppq (parts per quadrillion) range in breath samples. Further development of sampling devices will allow for at-home collection and identification of potential disease incidence prior to their first clinical visit.

Conclusion
Breath sampling offers a non-invasive, user friendly alternative to faecal sampling. It provides the same ability to detect gut microbial-produced VOCs, but with an easier and more standardised sample collection process, for use in both research and potential products for gastrointestinal disease detection. With applications ranging from diagnostics of IBD using metagenomics analysis to identifying patients most likely to respond positively in clinical trials, there is a clear demand for a highly sensitive and accurate breath sampling device to monitor metabolic activity.

REFERENCES

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