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ORIGINAL ARTICLE

Pilot study of a breath test for volatile organic compounds associated with oral malodor: evidence for the role of oxidative stress

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BACKGROUND: We performed a pilot study of a new method to identify the volatile organic compounds (VOCs) in breath associated with oral malodor, using gas chromatography and mass spectroscopy (GC/MS).

METHODS: Oral cavity breath was collected from seven patients with oral malodor. Breath samples (150 ml) were concentrated onto sorbent traps and analyzed by GC/MS.

RESULTS: Organoleptic scores ranged from 3.0 to 4.0 (mean = 3.3) on a scale of 0–5. Twenty-four of 30 (80.0%) of the most abundant oral malodor volatile organic compounds (OMVOCs) were alkanes and methylated alkanes. These VOCs are products of oxidative stress, generated by lipid peroxidation of polyunsaturated fatty acids in cell membranes.

CONCLUSIONS: Increased oxidative stress in the oral cavity of patients with oral malodor may account for the increased risk of atherosclerosis, coronary heart disease and stroke associated with periodontal disease. The breath test for OMVOCs could potentially provide an objective new test for the assessment of oral malodor.
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Keywords: volatile organic compounds; GCMS; oral malodor; breath test

Introduction

Oral malodor is a common disorder, often associated with bacterial infections of the periodontal area and the dorsum of the tongue (Figueiredo *et al*, 2002). The intensity of oral malodor may be ranked by expert odor judges employing an organoleptic scale, or with assays of volatile organic compounds (VOCs) in breath, e.g. sulfur-containing compounds (Scully *et al*, 1997). We report here the findings of a sensitive new method employing gas chromatography and mass spectroscopy (GC/MS) for detecting a wide spectrum of oral malodor volatile organic compounds (OMVOCs). This was a pilot study focused principally on methods development, in order to evaluate the feasibility of a new analytical method prior to evaluating it in a larger scale study.

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Materials and methods

Human subjects

Seven subjects were recruited with a history of oral malodor. Two trained organoleptic judges independently scored their severity of oral malodor using the following scale: 0, no odor present; 1, barely noticeable odor; 2, slight but clearly noticeable odor; 3, moderate odor; 4, strong odor; 5, extremely strong odor. Human research was approved by the Institutional Review Board of Saint Vincent Catholic Medical Centers, New York, NY, USA and the Biomedical Institute of America, San Diego, CA, USA.

Breath test for OMVOCs

All subjects donated a sample of oral cavity breath by inflating a multi-laminate 200 ml breath collection bag (Quintron, Inc., Milwaukee, WI, USA). Bags were heated to 38°C, and breath samples (150 ml) were extracted and injected onto sorbent traps (Carbotrap, Supelco, Bellefonte, PA, USA) in order to capture VOCs. Samples were assayed by GC/MS using a reported method (Phillips *et al*, 1999). VOCs were identified by a computer-based library and quantified by their ratio to an internal standard. Background VOC contaminants in the breath collection bags were determined by similar assays of bags inflated with ultra-pure helium, and subtracted from VOCs in breath.

Results

Organoleptic scores ranged from 3.0 to 4.0, mean = 3.3. The 30 most abundant VOCs in oral cavity breath are shown in Table 1. An unexpected finding was that the majority of these VOCs (24/30, 80%) were alkanes or alkane derivatives (displayed in Figure 1).

Table 1 Most abundant VOCs in breath of patients with oral malodor

Breath VOC	*Mean*
Benzene, methyl-	8.25
Butane, 2,2,3,3-tetramethyl-	4.75
Ethanol	4.64
Hexane, 2,2,5-trimethyl-	4.09
1-Propene, 2-methyl-	4
1,3-Butadiene, 2-methyl-	3.87
Nonane, 3-methyl-5-propyl-	3.59
Decane, 2,2-dimethyl-	3.42
Hexane, 3-methyl-	3.18
Cyclopentane, methyl-	2.94
Hexane	2.84
Cyclohexane, methyl-	2.63
Hexane, 2-methyl-	2.63
Cyclohexane	2
Pentane, 2,3-dimethyl-	1.5
Undecane, 3-methyl-	1.38
Butane, 2-methyl-	1.21
2-Butanone	1.11
Pentane, 3-methyl-	1.01
Heptane	0.96
Pentane, 3-ethyl-2,2-dimethyl-	0.82
Decane, 2,2,8-trimethyl-	0.75
Pentane, 2,3,3-trimethyl-	0.75
Pentane, 2-methyl-	0.75
Pentane, 2,3,4-trimethyl-	0.72
Hexane, 2,2,4-trimethyl-	0.57
Pentane	0.55
Acetaldehyde	0.55
Cyclopentane, ethyl-	0.54
Hexane, 2,2,3-trimethyl-	0.53

The 30 most abundant VOCs are ranked by relative abundance (mean value of ratio to abundance of an internal standard). VOCs shown in bold script are alkanes or methylated alkanes.

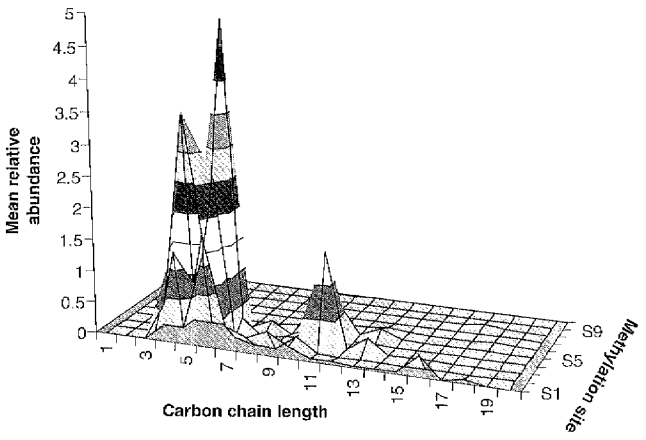

Figure 1 Oral malodor volatile organic compounds (OMVOCs) generated by oxidative stress. This figure displays the mean abundance of alkanes and monomethylated alkanes in the oral cavity breath of seven subjects with oral malodor. These VOCs are products of oxidative stress which are generated by lipid peroxidation of membrane polyunsaturated fatty acids. The carbon chain length is shown on the *x*-axis, ranging from C4 (butane) to C20 (eicosane), and the methylation site is shown on the *z*-axis. The abundance is shown on the *y*-axis

Discussion

We have previously reported the composition of VOCs in alveolar breath employing a different collection method, but a similar analytical method (Phillips *et al*, 1999). A study of 50 normal subjects revealed approximately 200 VOCs in each sample of breath, and more than 3000 different VOCs in the entire group. A very wide spectrum of chemical compounds was observed in alveolar breath, and it is possible that oral breath may also harbor a similar diversity of VOCs. However, one limitation of the collection method is that the adsorptive material in the sorbent trap selectively captures VOCs with two carbon atoms or more, so it is possible that lower molecular weight VOCs (e.g. sulfur compounds such as H_2S) may be underestimated in this assay.

The alkanes and alkane derivatives observed in oral cavity breath are known to be products of oxidative stress, in which mitochondria produce excessive quantities of reactive oxygen species that leak into the cytoplasm and oxidize several biologically important molecules, including DNA, lipids, carbohydrates and proteins (Therond *et al*, 2000). Lipid peroxidation of polyunsaturated fatty acids generates peroxyl radical which decomposes to aldehydes and alkanes (Dotan *et al*, 2004; Kneepkens *et al*, 1992). The abundance of volatile alkanes and methylated alkanes in the breath varies with the intensity of oxidative stress (Phillips *et al*, 2000).

Increased oxidative stress in the oral cavity of patients with oral malodor carries important clinical implications. Oral malodor is usually a consequence of infection in the oral cavity (Bosy, 1997), and periodontal infection has been linked with increased oxidative stress (Sculley and Langley-Evans, 2003; Takane *et al*, 2002). Periodontal disease has also been linked with an increased risk of atherosclerosis (Haynes and Stanford, 2003), coronary heart disease (Lopez *et al*, 2002) and stroke (Grau *et al*, 2004). These observations may be causally linked: it is possible that a focus of oral infection (e.g. gingivitis or periodontitis) generates increased oxidative stress, resulting in increased oxidation of LDL-cholesterol and accelerated atherosclerosis, thereby increasing the risk of coronary heart disease and stroke (Weinbrenner *et al*, 2003). The abundance of these VOC markers of oxidative stress was dramatically higher on oral breath than in previous observations of alveolar breath in normal subjects. However, correlation of oxidative stress with the intensity of oral malodor will require a larger future study that permits comparison of subjects with high and low levels of oral malodor.

These findings suggest that oral malodor may be considerably more serious than a social embarrassment – it may also be a sign of increased oxidative stress in the oral cavity and carry an increased risk of life-threatening vascular disease. This test for OMVOCs could potentially provide an objective new test for the assessment of oral malodor, and a larger clinical study is in progress to test this hypothesis.

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