Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease


Volatile sulfur compounds (VSC) in mouth air were estimated by gas chromatography. The amount of VSC and the methyl mercaptan/hydrogen sulfide ratio were significantly increased in patients with periodontal disease. These two parameters also increased in proportion to the bleeding index and probing depth. A study was also done on the effect of removal of tongue coating on VSC concentrations in mouth air from patients with periodontal involvement. VSC and the methyl mercaptan/hydrogen sulfide ratio were reduced to 49% and 35%, respectively, by removal of the tongue coating. The average amount of tongue coating removed from patients with periodontal disease was significantly higher than from controls (90.1 mg vs. 14.6 mg, p < 0.01). Estimated production of VSC from tongue coating was 4 times higher than the control value, and the methyl mercaptan/hydrogen sulfide ratio was also markedly increased. However, a saliva putrefaction study suggested that saliva does not contribute to the elevated ratio of methyl mercaptan in mouth air. These results strongly suggest that, in addition to periodontal pockets, tongue coating has an important role in VSC production, in particular leading to an elevated concentration of methyl mercaptan, which is more pathogenic than hydrogen sulfide.

Key words: volatile sulfur compounds — tongue coating — saliva — periodontal disease

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Introduction

Patients with periodontal disease frequently suffer from oral malodor, which is caused mainly by volatile sulfur compounds (VSC) such as hydrogen sulfide, methyl mercaptan and dimethyl sulfide (1, 2). VSC are produced through the putrefactive activities of microorganisms in saliva, the gingival crevice, the tongue surface and other areas (3, 4, 5).

An early study (6) demonstrated that periodontal disease gives rise to an unpleasant odor that is reflected in mouth odor intensity. Rizzo (7) found that the highest concentration of hydrogen sulfide occurred in the deepest pockets. Tonzetich (8) observed a positive correlation between the severity of periodontitis and VSC content in mouth air. Also Tonzetich and McBride (9) described copious production of methyl mercaptan by periodontally pathogenic microorganisms. On the other hand, it has been suggested that VSC (10–16), especially methyl mercaptan (10, 16), accelerate periodontal disease, because methyl mercaptan had a more pronounced effect on the permeability of oral mucosa than did a similar concentration of hydrogen sulfide (10). Furthermore, as sulfides are more cytotoxic than thiols (17) and as methyl mercaptan can be dimerized to dimethyl sulfide, methyl mercaptan is considered to be more cytotoxic than hydrogen sulfide, although this compound may not be a primary periodontal pathogen. However, if methyl mercaptan is present at high concentrations in mouth air from patients with periodontal disease, this might accelerate the progress of this disease secondarily. But no comprehensive data are available on the content of methyl mercaptan in mouth air from patients with periodontal disease.

Periodontal pockets are believed to be the main site of VSC production in affected patients (8, 9, 18, 19). It has been postulated that the tongue coating may not play an important role in the production of VSC in periodontal disease (20), whereas removal of tongue coating does reduce VSC in orally healthy subjects (18, 20). However, a preliminary study has suggested that the volume of tongue coating tends to increase in case of perio-
dental involvement (21). Therefore, if copious production of methyl mercaptan occurs in periodontal disease, the tongue coating might contribute to this.

In the present study we investigated the composition of VSC in mouth air to assess whether copious production of methyl mercaptan occurs in periodontal disease. An attempt was also made to determine the source of VSC production employing periodontal examination, a saliva putrefactive system and examinations of the tongue coating.

Material and Methods

VSC analysis of mouth air and collection of samples

Thirty-one subjects (mean age 34 years; 15 males, 16 females) were instructed to abstain from oral hygiene, including oral rinsing, and ingestion of food and liquid on the morning of the test. Experiments were performed between 8:30 and 9:30 a.m. The VSC analysis system included a GC-8A gas chromatograph (Shimadzu, Japan) equipped with a flame photometric detector, a 12-ft × 1/8 inch Teflon column packed with 5% polyphenyl ether-0.05% phosphoric acid on 40/60 mesh Chromosorb T, and an auto-injection system with a 10-ml or 3-ml sample loop to avoid strong back pressure at the injection port.

Prior to each analysis, the subject was instructed to keep the mouth closed and to breathe through the nose for 1 min. Then the Teflon tube connected to the auto-injector was inserted into the center of the oral cavity through the lips and teeth, while the mouth remained closed. Following aspiration of 15 ml of mouth air with the syringe connected to the outlet of the auto-injector, a 10-ml sample of air was transferred to the column and chromatographed. Column conditions were as follows: column temperature 70°C, nitrogen gas flow rate 32 ml/min, hydrogen gas flow rate 125 ml/min, air flow rate 43 ml/min. For each subject, two analyses were done at the same time, and then the average amount of each VSC was calculated.

After VSC analysis, each participant donated a sample of paraffin-stimulated whole saliva, from which food debris was then removed using two layers of cheese-cloth. Then the saliva was subjected to the saliva putrefaction study. After saliva collection, the tongue coating was removed from each subject as follows: First, cotton rolls were put around the tongue to exclude moisture, then saliva on the dorsal surface of the tongue was removed with a stream of air and pure pulp tissue paper (Kim Wipe, USA). The tongue coating was then carefully removed with a tongue scraper of the small spoon type, developed by the author (K.Y.), from the terminal sulcus to the apex of the tongue, then the tongue dorsal surface was cleaned with cotton pellets immersed in physiologic saline. After removal of the tongue coating, VSC analyses of mouth air were repeated and the wet weight of the tongue coating was estimated (mg). Unlike previous studies of tongue coating (20–22), which have usually employed classification by inspection for estimating the volume of tongue coating, the present investigation objectively estimated the actual amount of tongue coating removed.

The concentrations of VSC were expressed as nanograms per 10 ml volume of mouth air, and total sulfur (ng/10 ml air) was derived from the sulfur equivalents of all the VSC concentrations.

Saliva putrefaction

Immediately after collection of saliva, 1-ml aliquots of saliva were transferred to individual Teflon-coated glass tubes (17.5 ml volume). The tubes were capped with a solid rubber disk lined with Teflon film to provide an airtight seal. The Teflon coating prevented glass or rubber from coming into contact with the head-space air.

The head-space air in the tube was replaced with nitrogen gas to simulate a relatively anaerobic condition, then incubation was carried out for 24 h at 37°C without shaking. For analysis, the head-space air was processed as follows: A Teflon-coated injection needle, which was connected to the VSC analysis system, was used to withdraw a sample of head-space air from each tube by insertion of the needle through a 2-mm diameter hole in the airtight plastic cap. Then 4 ml of nitrogen was injected into the tube, and the same volume of head-space air was aspirated. Three-milliliter samples were injected into the VSC analysis system.

Periodontal evaluation

Standardized pocket depth measurements (23) were made at three points on the buccal and lingual sides of each tooth. Also the bleeding points caused by probing were counted, and bleeding index (23), which is the ratio of the number of bleeding points to the number of probings, was obtained.

Results

VSC in periodontal disease

VSC concentrations in mouth air from patients with probing depth of 4 mm or more were demonstrated to be higher than in subjects with a probing depth below 4 mm. In particular, the methyl mercaptan concentration was significantly higher than in controls (44.0 ng/10 ml vs. 2.6 ng/10 ml, p < 0.01) (Fig. 1). Also the methyl mercaptan/hydrogen sul-
Volatile sulfur and periodontal disease

Fig. 1. VSC production in mouth air from subjects (n = 17) with a gingival probing depth of 4 mm or more. In comparison with subjects (n = 14) with a probing depth of less than 4 mm, a large amount of VSC production was observed, methyl mercaptan ratio being markedly increased.

Bleeding as a result of probing was also examined. Fig. 2 shows that VSC concentrations were higher in mouth air from patients with bleeding points caused by probing. Methyl mercaptan was markedly increased in these patients in comparison with hydrogen sulfide (methyl mercaptan; 38.1 ng/10 ml vs. 23.0 ng/10 ml, hydrogen sulfide; 6.6 ng/10 ml vs. 2.1 ng/10 ml). Also the methyl mercaptan/hydrogen sulfide ratio in subjects with a probing depth of 4 mm or more was significantly higher than in controls, as shown in Fig. 2 (4.1 vs. 0.5, p < 0.01).

Bleeding index was used to compare the extent or severity of periodontitis. Fig. 3 demonstrates that total sulfur content and the methyl mercaptan/hydrogen sulfide ratio increased with bleeding index, thereby indicating that VSC production and the methyl mercaptan ratio increased with the extent of periodontal disease.

The maximum depth of probing in each patient was determined, and compared with the methyl mercaptan/hydrogen sulfide ratio. The results showed that the ratio increased with probing depth (Fig. 4). The group with a probing depth of 3 mm or less had an average ratio of 0.37 ± 0.10 (mean ± SE, n = 9); for a 4-mm probing depth the
Table 1. Effect of removal of tongue coating on VSC production

<table>
<thead>
<tr>
<th>Removal of Tongue Coating</th>
<th>Total Sulfur (ng/10 ml)</th>
<th>CH₃SH/H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;4 mm*</td>
<td>≥4 mm*</td>
</tr>
<tr>
<td>Before</td>
<td>8.3±4.0</td>
<td>36.5±12.0</td>
</tr>
<tr>
<td>After (% reduction)</td>
<td>(51.8±12.8)</td>
<td>(49.0±16.4)</td>
</tr>
</tbody>
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*probing depth. mean ± SE.

gen sulfide ratio was much higher than in controls (31.3 vs. 1.0, p < 0.01). These results demonstrate that much more VSC, specially methyl mercaptan, is produced on the tongue dorsal surface in periodontal disease. To examine the effect of salivary flow on the amount of tongue coating, whole unstimulated salivary secretion was determined for 10 min. However, there was no large difference of flow rate between the patients with periodontal disease and healthy controls (2.4±0.6 ml vs. 2.9±0.6 ml, mean ± SE), and no relation between tongue coating and the salivary flow rate. These observations indicate that salivary flow rate does not affect the accumulation of tongue coating in patients with periodontal disease.

Table 2. VSC production from the tongue coating and amount of tongue coating

<table>
<thead>
<tr>
<th>Probing Depth</th>
<th>Wet Weight (mg)</th>
<th>VSC (ng/10 ml)</th>
<th>CH₃SH/H₂S Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4 mm (n=14)</td>
<td>14.6±7.5</td>
<td>4.3±3.1</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>≥4 mm (n=17)</td>
<td>90.1±11.0</td>
<td>18.6±6.8</td>
<td>31.3±18.1</td>
</tr>
</tbody>
</table>

mean ± SE.

Fig. 5. VSC production in a saliva putrefaction system. VSC production in saliva from subjects (n=17) with a probing depth of 4 mm or over is almost double that of the control (n=14), but the ratio of methyl mercaptan is the same as the control average.
Discussion

Periodontal disease frequently gives rise to oral malodor (1, 20), and the intensity of the odor increases with the severity of periodontal disease, as described previously (8, 20, 24).

Tonzetich and Richter (1) were the first to report that VSC are the main components of oral malodor, contrary to the traditional belief that amine and ammonia are the most important sources. Thereafter, Tonzetich (2) developed an accurate method for determining VSC by gas chromatography. Recently, simple and compact detectors of halitosis have been developed employing semiconductor gas sensors, and these are becoming popular in the clinical field (25). However, since we have found that these detectors are sometimes unreliable in comparison with the results obtained by gas chromatography (unpublished data), a gas chromatography method established by Tonzetich (2) was employed in this study to carry out precise and detailed analysis of VSC.

VSC comprise hydrogen sulfide, methyl mercaptan and dimethyl sulfide. Since dimethyl sulfide is usually present only in trace amounts, hydrogen sulfide and methyl mercaptan, the main components of VSC in mouth air, were investigated in this study.

The results revealed a greater amount of VSC production and a higher methyl mercaptan/hydrogen sulfide ratio in subjects with a probing depth of 4 mm or more. Therefore these results strongly indicate that methyl mercaptan is the main component of VSC in patient with periodontal involvement, whereas slightly more hydrogen sulfide than methyl mercaptan is formed in orally healthy subjects.

Progression of periodontal pockets enhances the production of VSC (8, 18), and periodontally pathogenic strains of microorganisms accelerate the production of methyl mercaptan in particular (9). Coil and Tonzetich (18) suggested that the amount of methyl mercaptan produced may reflect the number and virulence of Porphyromonas gingivalis, which has been implicated in the etiology of periodontal disease. However, the mechanism of VSC production in periodontal disease is complicated and may also involve other clinical factors. There are other plausible reasons for the increased VSC in periodontal disease (8). Our study demonstrated that VSC concentrations and the methyl mercaptan/hydrogen sulfide ratio increased with bleeding index. Therefore it is suggested that some blood components in the oral cavity or periodontal pockets may accelerate VSC production. Gibbons and McDonald (26) found that most strains of Prevotella melaninogenica, including some periodontally pathogenic strains, required hemin for growth, and that the growth rate increased with hemin concentration.

On the other hand, tongue coating is believed to be the main source of VSC production in orally healthy subjects (8, 20). Tongue coating comprises desquamated epithelial cells, blood cells and bacteria (20). More than 100 bacteria may be attached to a single epithelial cell desquamated from the tongue dorsum, whereas only about 25 bacteria are attached to each cell in other areas of the oral mucosa (27). Kaizu (20) described the volume of tongue coating in subjects with oral malodor as significantly higher than in controls. A tongue coating putrefaction study (28) indicated that tongue coating has the potential to produce a large amount of VSC. It is known that removal of tongue coating reduces VSC production in mouth air from orally healthy subjects without periodontal or gingival disease (12, 20). However Kaizu (20) found that tongue coating removal is not effective for prolonging the time of suppression of methyl mercaptan production in patients with periodontal disease. This implies that tongue coating in periodontal disease cannot be a major source of VSC production. However, we demonstrated that removal of tongue coating does in fact considerably reduce the amount of VSC in periodontal disease, and that tongue coating in the oral cavity produces much more methyl mercaptan than hydrogen sulfide. Therefore, it is suggested that tongue coating as well as plaque in periodontal pockets is one of the main sources of VSC production in periodontally diseased patients and plays an important role in accelerating methyl mercaptan production in the oral cavity.

The concentrations of VSC in head space air of incubated saliva from patients were almost double those from orally healthy subjects. Yaegaki (29) described one reason for the elevated VSC production in saliva putrefaction as being that the disulfide content of saliva increases with the severity of periodontal disease. Therefore, it is postulated that saliva contains a higher concentration of hydrogen sulfide precursors which may accelerate VSC production. However, our results indicated that the methyl mercaptan/hydrogen sulfide ratio in head space air is not different between subjects with periodontal disease and orally healthy subjects. Therefore, although the elevated putrefaction activity of saliva promotes VSC production in periodontal disease, its contribution to the increased methyl mercaptan ratio is less than that of other factors such as tongue coating.

The present study strongly indicates that tongue coating and periodontal pockets containing blood components and bacteria may play an important
role in the production of methyl mercaptan in periodontal disease. The elevated concentration of methyl mercaptan may accelerate the progress of periodontal disease, rather than hydrogen sulfide, because methyl mercaptan is present at high concentrations in patients with periodontal disease.

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