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 purification 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.

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. Tonzeitch (8) observed a positive correlation between the severity of periodontitis and VSC content in mouth air. Also Tonzeitch 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 peri-
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, and methyl mercaptan ratio being markedly increased.

Fig. 2. VSC production in mouth air from subjects (n = 20) with bleeding points after probing. In comparison with the subjects (n = 11) without bleeding points, marked VSC production was observed, and the methyl mercaptan ratio was notably 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>CH$_3$SH/H$_2$S</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</td>
<td>4.0 ± 1.6</td>
<td>18.6 ± 6.8</td>
</tr>
<tr>
<td>(% reduction)</td>
<td>(51.8 ± 12.8)</td>
<td>(49.0 ± 16.4)</td>
</tr>
</tbody>
</table>

$^*$Probing depth. mean = SE.

Ratio was 0.96 ± 0.23 (mean ± SE, n = 6), 3.49 ± 1.48 (mean ± SE, n = 8) for a 5-mm depth and 6.10 ± 1.71 (mean ± SE, n = 11) for a depth over 5-mm. This demonstrated that the methyl mercaptan/hydrogen sulfide ratio increased with the severity of periodontal disease.

Effect of tongue coating on VSC production

Table 1 demonstrates the effect of tongue cleaning on VSC production and the methyl mercaptan/hydrogen sulfide ratio. Immediately after tongue cleaning, total sulfur production decreased by 51.8% in the group with a probing depth of less than 4 mm, and by 49.0% in patients with periodontal disease with a probing depth of 4 mm or more. Also, the methyl mercaptan/hydrogen sulfide ratio decreased by 55.5% in controls and 34.8% in periodontal disease patients. This suggested that removal of tongue coating markedly reduced both VSC production and the methyl mercaptan ratio, not only in orally healthy subjects but also in patients with periodontal disease.

The amount of tongue coating (wet weight) and VSC production from it were also determined. VSC production from the tongue coating was calculated by subtracting the amount of VSC immediately after tongue cleaning from the initial amount before cleaning, since the coating of only the dorsal surface of the tongue was carried out accurately without oral rinsing or tooth brushing. Table 2 showed that the group with periodontal disease (≥4 mm probing depth) had much more tongue coating than the control (14.6 mg vs. 90.1 mg, p < 0.01). VSC production from the tongue coating in periodontal disease patients was estimated to be more than 4 times that of controls (≤4 mm probing depth). However, the methyl mercaptan/hydrogen 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 saliva secretion was determined for 10 min. However, there was no large difference in 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.

Saliva putrefaction and VSC production

To determine the contribution of saliva to VSC production, a saliva putrefaction study was carried out. Fig. 5 shows that a large amount of VSC was produced in head space air from saliva of patients with periodontal involvement (≥4 mm probing depth). However, there was no significant difference between the methyl mercaptan/hydrogen sulfide ratios from these two groups.

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>CH$_3$SH/H$_2$S 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 amines 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 constituents 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 patients 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): C. alveolar 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. Thereby, 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. Yagaki (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.

Acknowledgment

We are very grateful to Dr. Hasegawa, Professor, Dr. Hamaguchi, and Dr. Sakai, Department of Periodontics, School of Dentistry at Niigata, The Nippon Dental University, for their assistance.

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