The Relationship Between Oral Malodor, Gingivitis, and Periodontitis. A Review*

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Volatile sulfur compounds (VSC) are a family of gases which are primarily responsible for halitosis, a condition in which objectionable odors are present in mouth air. Although most patients perceive this condition as primarily a cosmetic problem, an increasing volume of evidence is demonstrating that extremely low concentrations of many of these compounds are highly toxic to tissues. VSC may, therefore, play a role in the pathogenesis of inflammatory conditions such as periodontitis. Since these compounds result from bacterial putrefaction of protein, investigations have been conducted to determine whether specific bacteria are associated with odor production. Two members of this family, hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH), are primarily responsible for mouth odor. Although many bacteria produce H_2S, the production of CH_3SH, especially at high levels, is primarily restricted to periodontal pathogens. Direct exposure to either of these metabolites adversely affects protein synthesis by human gingival fibroblasts in culture. However, methyl mercaptan has the greatest effect. Other in vitro experiments have demonstrated that cells exposed to methyl mercaptan synthesize less collagen, degrade more collagen, and accumulate collagen precursors which are poorly cross-linked and susceptible to proteolysis. CH_3SH also increases permeability of intact mucosa and stimulates production of cytokines which have been associated with periodontal disease. VSC, and in particular methyl mercaptan, are therefore capable of inducing deleterious changes in both the extracellular matrix and the local immune response of periodontal tissues to plaque antigens. This article reviews these data and emphasizes the potential importance of VSC in the transition of periodontal tissues from clinical health to gingivitis and then to periodontitis. *J Periodontol 1999;70:485-489.*

KEY WORDS

Extracellular matrix; gingivitis/pathogenesis; halitosis/pathogenesis; periodontitis/pathogenesis; hydrogen sulfide/toxicity; methyl mercaptan/toxicity; immune response.

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Oral malodor can be attributed to a variety of products arising from bacterial metabolism of amino acids. These metabolites include many compounds such as indole, skatole, and volatile sulfur compounds (VSC), hydrogen sulfide, methyl mercaptan, and dimethyl sulfide.[1] VSC have been shown to be the main cause of human oral malodor.[2] Studies have also demonstrated that these compounds are toxic at low concentrations.[3] VSC may, therefore, not only be associated with oral malodor but also probably contribute to the etiology of both gingivitis and periodontitis.

Periodontal disease results from the combination of many factors present in vivo. These processes include chronic activation of the immune system, alterations in connective tissue metabolism, production of proteinases and cytokines, direct destruction of host tissues by bacterial enzymes, and virulence factors and a multitude of other mechanisms.[4] The disease process is, therefore, not necessarily a sequential series of events but rather a consequence of concurrent processes which act in concert to produce destruction of tissues.

PRINCIPAL CAUSE OF ORAL MALODOR IN HEALTH

VSC most frequently associated with oral malodor are hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH).

Other compounds such as dimethyl sulfide (CH_3SCH_3) and dimethyl disulfide (CH_3SSCH_3) are also members of this family and can be detected in mouth air of some patients but are not normally present at levels which would be considered objectionable.[5] Both H_2S and CH_3SH contain free thiols (-SH groups) which have the potential to chemically react with both DNA and proteins.

VSC have been shown to result from the bacterial putrefaction of proteins with sulfur-containing amino acids. These proteins are derived from exfoliated human epithelial cells and white blood cell debris. There are no data on the role of VSC formation from putrefied foods, such as hamburger. In other words, the known substrate required for the formation of VSC originates from host tissues. In addition, the production of VSC has been shown to be significantly increased by the presence of pooled blood.[6]

The two main anatomical sources of VSC which have been identified in the oral cavity are the gingival sulcus and the tongue.[7] Although the tongue is the principal source of oral malodor, VSC derived from the gingival sulcus also contribute thiols to mouth air. In addition, the relative proportions of hydrogen sulfide and methyl mercaptan produced by each of these sites appear significantly different.[8]

In contrast to VSC in mouth air where H_2S is present in the greatest concentration, measurements of VSC within periodontal pockets have demonstrated that CH_3SH is often the predominant compound. Persson et al.[9-11] found that the ratios of CH_3SH/H_2S were moderately increased in deeper pockets. However, increases in these ratios were greatest and most highly correlated with disease when pockets were segregated using presence or absence of bleeding on probing. These data would indicate that the presence of methyl mercaptan within a periodontal pocket may be associated with active periodontal disease.

Specific bacteria identified in the production of VSC have been reported in both the periodontal literature and a doctoral thesis by Persson et al.[9-11] Many of these bacteria are commonly suspected periodontal pathogens. Persson found that 82 bacterial species form H_2S from heat-inactivated serum and 25 bacterial strains form CH_3SH from heat-inactivated serum. Twelve subspecies of *F. nucleatum* produced both H_2S and CH_3SH. There are several reports studying VSC-producing bacteria,[12-16] and a review of the literature on oral malodor was published in 1990 by Kleinberg and Westbay.[17]

TRANSITION FROM HEALTH TO GINGIVITIS

While the preceding events continue, a new group of cellular changes may occur, producing gingivitis. Gingivitis is characterized by an immune response to antigens in bacterial plaque as well as by alterations in connective tissue. One of the earliest events associated with disease is enhanced permeability of the lining epithelium within the gingival sulcus. Bacterial antigens such as lipopolysaccharide (LPS) induce gingival inflammation in some individuals but mere exposure to these antigens is not necessarily sufficient to cause gingivitis in all patients.[4]

VSC are potentially capable of altering permeability of the gingival tissues, inducing inflammatory responses, and modulating functions of gingival fibroblasts (Fig. 1). Early work by Rizzo[18] indicated that a facilitating agent is required to allow LPS to penetrate healthy gingival epithelium and subsequently initiate an inflammatory response. Although no inflammatory response could be initiated by topical application of LPS to healthy gingiva, exposure of these tissues to H_2S facilitated penetration of LPS and resulted in inflammation.[9]

A subsequent study by Ng and Tonzetich[19] reported that exposure of sublingual porcine tissue to either H_2S or CH_3SH for times of up to 3 hours increased penetration of the tissues to $[^{35}S]$-Na_2SO_4. In addition, these experiments showed that $[^{35}S]$-H_2S penetrated and was retained by the tissue slices.[19] VSC have also been shown to penetrate deeply into the tissues where they can induce deleterious changes in the non-keratinized epithelium, basement membrane, and underlying lamina propria which can be demonstrated using histologic sections.[20]

These studies demonstrated not only that VSC can alter non-keratinized soft tissues, but also that these compounds can cause these changes at low concentrations and within short periods of time. Also, VSC are not only directly toxic to tissues, but they may also facilitate entry of other bacterial antigens, such as LPS, into the underlying lamina propria. These data are consistent with the hypothesis that thiols participate in early stages of the inflammatory response and may be important initiators of gingivitis.

When inflammation occurs in the gingiva, the focus of change is in the connective tissue area. However, initial changes in the epithelium are likely important early events in the progression of periodontal disease. Epithelial cells in an inflamed environment will produce collagenase.[21-23]

Epithelium has no vascular supply and is totally dependent upon the available nutrients and oxygen from the underlying connective tissue. Tritiated thymidine marker studies have indicated that both gingivitis and periodontitis induce an 8-fold increase in cell mitosis.[24] These observations would predict that this increased rate of cell division should require a substantial nutrient base which may not be available due to the venous stasis in the inflamed connective tissue. The epithelium may, therefore, exhibit an initial increase in permeability associated with factors such as alterations

in the extracellular matrix between cells. However, the epithelial ulceration associated with periodontal disease is more likely caused by events such as vascular stasis in the underlying connective tissue leading to the subsequent necrosis of epithelial cells. The main result of this ulceration is that it provides a portal of entry for bacterial and larger bacterial metabolites into the underlying lamina propria. At present, the effects of VSC on the physiology of blood vessels have not been studied and are an important area for future research.

Gingivitis results from the induction of an immune response accompanied by alterations in fibroblast function. CH_3SH has been shown to induce secretion of interleukin-1 beta (IL-1ß) from mononuclear cells in culture.[25] IL-1ß has been isolated from inflamed gingival tissue and may play a significant role in the pathogenesis of periodontal disease. Methyl mercaptan has also been shown to act synergistically with both LPS and IL-1ß to increase secretion of prostaglandin E_2 and collagenase, important mediators of inflammation and tissue destruction.[25]

VSC have direct effects on the formation of extracellular matrix by human gingival fibroblasts in culture. Experiments have demonstrated that exposure to either H_2S or CH_3SH for between 24 and 48 hours lowers total protein production by these cells. In addition, although both thiol compounds lower protein content, methyl mercaptan has the greater effect.[3] Methyl mercaptan has been shown to inhibit synthesis of collagen as well.[26] The concentrations of thiols employed in these studies (2 ppm) were slightly higher than normally seen in mouth air of periodontal patients (0.5 ppm). However, since concentrations detected in mouth air are diluted by ambient air, the higher amounts used in these experiments are likely comparable to levels in periodontal pockets.

The effects of CH_3SH on collagen metabolism are a reflection of both decreased synthesis and increased degradation of protein. Hydroxyproline analysis of mercaptan-exposed fibroblasts has demonstrated that both aspects of collagen metabolism are affected. In addition, the increased degradation is likely associated with inhibition of procollagen peptidase enzymes which are essential for procollagen processing since elevated amounts of procollagens can be demonstrated in CH_3SH-exposed gingival fibroblast cultures. Since procollagens will not efficiently cross-link and form mature collagen fibrils, these immature collagens are likely susceptible to enzymatic degradations. Inhibition of procollagen peptidases would therefore affect both synthesis and degradation of collagens.[26]

The effects of both CH_3SH and H_2S on proteins likely result from the inherent reactivity of the thiol (-SH) group in both of these compounds. Previous studies with type I collagen have demonstrated that both of these gases, when labeled with radioactive sulfur^{35}, will bind to collagen and that a significant amount of the sulfur incorporated into the protein is tightly bound (likely through covalent binding). Dimethyl disulfide, a VSC which does not have a reactive thiol, is essentially inert.[27]

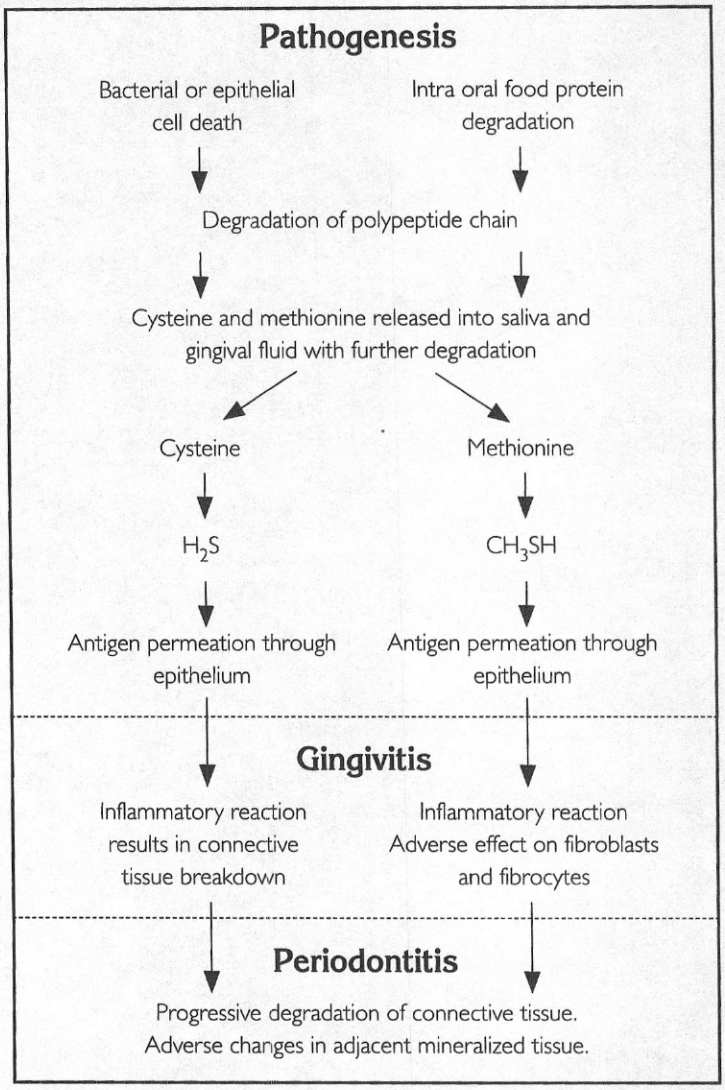

Figure 1.
Effects of volatile sulfur compounds which may potentiate gingivitis and periodontitis.

THE TRANSITION FROM GINGIVITIS TO PERIODONTITIS

In the change from gingivitis to periodontitis, there is a continuation of all the events in the oral malodor and gingivitis sections as well as a new group of events that occur in the development of periodontitis. Periodontitis results from destruction of both the hard and soft tissue structures which support teeth. The transition from gingivitis to periodontitis is mainly an anatomical difference in which the disease progresses into the underlying bone. These histologic observations do not, however, represent differences in the basic etiologic factors which have initiated the alterations in the overly-

ing soft tissues. The important consideration with respect to periodontitis is, therefore, the effects of these same factors on mineralized tissues as well as a combination of soft and mineralized tissues.

Since the periodontal ligament (PDL) cell is associated with the formation and maintenance of the mineralized supporting structures,[28] effects of thiols on these ligament cells are particularly relevant. Moreover, since the major extracellular matrix (ECM) protein in bone is type I collagen, alterations in collagen will likely have a dramatic effect on hard tissues. Experiments which have correlated increases in periodontal probing depth and bleeding on probing with increases in methyl mercaptan in these pockets are also relevant since they indicate that effects resulting from exposure to CH_3SH become increasingly important in periodontitis.[8] These observations are in accordance with results obtained from analysis of mouth air of periodontal patients which have demonstrated a correlation between increases in CH_3SH/H_2S concentration ratios and increases in periodontal pocket depth.[29]

Studies have shown that PDL cells exposed to methyl mercaptan in culture alter their intracellular pH and become more acidic. In addition, they exhibit decreased motility, lowered protein synthesis, and alterations in collagen metabolism. These changes are predominantly detrimental to the ability of these cells to maintain or regenerate mineralized tissues.[30] Results have indicated that exposure of PDL cells to CH_3SH results in changes in collagens which are much like those observed in gingival fibroblasts exposed to mercaptan. There appears to be a similar inhibition of procollagen peptidases resulting in accumulation of procollagen precursors. In addition, there are substantial reductions in amounts of type III collagens.[30] This observation is significant since periodontally involved tissues are known to exhibit substantial losses of type III collagens which decrease from 20% to 30% to 4% of total collagens.[31]

Other ECM proteins are also affected. Fibronectin in PDL cell cultures treated with mercaptan exhibits less disulfide-mediated cross-linking. These cells, rather than producing high-molecular weight multimers, produce fibronectin of lower molecular weight which corresponds to monomeric rather than the usual dimeric or multimeric protein. Since both collagen and fibronectin play important roles in cell migration, changes in these ECM molecules may contribute to the decreases in cell motility observed in PDL cell cultures exposed to CH_3SH.[32]

CLINICAL RELEVANCE

Treatment of oral malodor relates to 3 aspects of patient care. Therapeutic objectives may, therefore, encompass treatment for all or just some of these factors depending on the needs of individual patients. Compounds such as VSC play 1) a cosmetic role in social interactions; 2) a role as a facilitating agent which may accentuate the effects of other factors participating in early stages of gingivitis; and 3) a role as a pathologic agent which may contribute to the disease process directly. Therapy should, therefore, be determined by the individual needs of patients. For example, mouthwashes may treat the cosmetic aspects of oral malodor but cannot be considered as the only treatment required for patients with advanced periodontal disease.

Treatment of malodor should not be considered as just cosmetic therapy, since the available evidence indicates that many members of the VSC family are toxic to periodontal tissues even when present at extremely low concentrations. These data may be extremely important in the pathogenesis of periodontal disease since the thiols are present in a confined periodontal pocket, which allows their concentrations to accumulate near the sulcular epithelium. Periodontal tissues, unlike the tongue and alveolar mucosa, are not protected by a keratinized layer and may be particularly susceptible in injury. Traditional procedures of scaling, root planing and practice of oral hygiene combined with tongue scraping are effective at reducing levels of these compounds in mouth air and are satisfactory for cosmetic treatment. However, oral care products which can demonstrate efficacy at lowering concentrations of VSC in periodontal pockets may also be significant adjuncts to periodontal therapy as well as prevention of gingival disease.

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