

Literature Review

Volatile sulphur compounds elimination: A new insight in periodontal treatment

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ABSTRACT

Background: Recent evidences had demonstrated a link between halitosis and apoptosis in periodontitis. Periodontal pathogenic micro-organisms produce volatile sulphur compounds (VSCs). VSCs are toxic to periodontal tissue. **Purpose:** The purpose of this paper was to reveal the mechanism of VSCs in periodontal breakdown according to the most recent knowledges. **Reviews:** Halitosis is mainly attributed to VSCs such as hydrogen sulfide, methyl mercaptan and dimethyl sulfide. Several studies demonstrated a strong relationship between VSCs and periodontal disease progression. VSCs are released from amino acid breakdown from food, protein, cells, blood and saliva. In prone subjects, the VSCs may cause alteration in tissue integrity by increasing its permeability and facilitate the endotoxin to penetrate the tissue barrier. They may also causing apoptotic in gingival and periodontal tissue, which are considered the main pathogenesis in aggravating the periodontitis. VSCs may also initiate the increase of proinflammatory cytokines which is considered to have negative effects in host response. **Conclusion:** VSCs had been shown to have detrimental effects in gingival and periodontal ligament cells. The use of chlorine dioxide agent and topical antioxidant is beneficial in controlling the periodontal disease severity.

Key words: Periodontitis, volatile sulphur compounds (VSCs), apoptosis, chlorine dioxide

ABSTRAK

Latar belakang: Penelitian terakhir menunjukkan adanya hubungan antara halitosis dengan terjadinya apoptosis pada periodontitis. Mikroorganisme penyebab periodontitis memproduksi volatile sulphur compounds (VSCs) yang bersifat toksik terhadap jaringan periodontal. **Tujuan:** Tujuan penulisan ini adalah membahas mekanisme VSCs dalam menyebabkan kerusakan periodontal berdasarkan penelitian terakhir yang ada. **Tinjauan pustaka:** Halitosis seringkali dikaitkan dengan timbulnya VSCs seperti hidrogen sulfida, metil merkaptan, dan dimetil sulfida. Penelitian terakhir menunjukkan bahwa VSCs yang dilepaskan dari pemecahan asam amino makanan ternyata memiliki korelasi dengan kerusakan jaringan periodontal. Pada subjek yang peka, VSCs dapat menyebabkan terjadinya disintegritas epitel dengan meningkatkan permeabilitasnya sehingga endotoksin dapat masuk melewati epitel. Adanya VSCs tersebut memicu terjadinya apoptosis pada jaringan gingiva dan ligamen periodontal, dimana proses ini menyebabkan keparahan penyakit periodontal. VSCs juga dapat mencetuskan peningkatan sitokin proinflamasi yang dapat menyebabkan kerusakan jaringan periodontal. **Kesimpulan:** VSCs dapat menyebabkan kerusakan pada gingiva dan ligamen periodontal. Penggunaan bahan chlorine dioxide untuk dan antioksidan topikal sangat berguna dalam mengontrol keparahan penyakit periodontal.

Kata kunci: Periodontitis, volatile sulphur compounds (VSCs), apoptosis, chlorine dioxide

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INTRODUCTION

Halitosis originated from 'halitus', the Latin word for breath, means a complaint analogous to body odor and is used to refer the offensive odor emanating from the oral cavity. With up to 50% of people worldwide assessing themselves as having frequent or constant incidents of malodor, it is a common complaint of many adults.¹ Halitosis usually affects interpersonal social communication and has also become an important market for the pharmacological and cosmetic industries. It is estimated that 20% of young male in Switzerland suffer from halitosis.² Another study had shown the percentage of halitosis ranged vary from 22–50%. According to the American Dental Association, 50% of the adult population had suffered from an occasional oral malodor disorder, while 25% seemed to have a chronic problem. Consequently, halitosis become a major sources of multimillion-dollar industry. In the USA alone, over US\$500 million are spent annually on mouthwashes, sprays, and related over-the-counter products toward to management of this common problem.^{3,4}

There are various compounds that produce unpleasant smelling in human oral environment, such as hydrogen sulfide, methyl mercaptan, dimethylsulfide, butyrate, isovalerate, skatole, trimethylamine, and putrescine. The hydrogen sulfide, methyl mercaptan and dimethyl sulfide, that arise from bacterial metabolism of aminoacids, mainly contribute to oral malodor. They comprise up to 90% the volatile sulphur compound (VSCs) content of mouth air and have been shown to increase with periodontal disease.⁵ It has been demonstrated that the intensity of clinical bad breath is significantly associated with amount of intra-oral VSCs level. The periodontal pocket is an ideal environment for VSCs production with respect to the bacterial profile and sulfur source. In addition, VSCs also accelerate in periodontal tissue destruction.⁶ This may explain why patients with periodontal diseases often complain of oral malodor. The purposes of this paper is to explain the mechanism of VSCs in aggravating periodontal disease.

Periodontal disease

Periodontal disease is one of the two major dental diseases that affect human populations worldwide at high prevalence rates. An advanced periodontal disease with deep periodontal pockets (6 mm or more) affects 10% to 15% of adults worldwide. The available evidence shows that important risk factors for periodontal disease relate to poor oral hygiene, tobacco use, excessive alcohol consumption, stress, and diabetes mellitus. Periodontitis is defined as an inflammatory disease of the supporting tissues of teeth caused by specific microorganism or group of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both. Periodontitis results in the formation of soft tissue pockets. Severe periodontitis can result in loosening of teeth, occasional pain and discomfort, impaired mastication, and eventual

tooth loss. The microflora of the mouth contains three hundreds of species of aerobic and anaerobic bacteria. These organisms grow on tooth surfaces as complex, mixed, interdependent colonies in biofilms, and are attached and densely packed against the tooth in the deeper layers, with more motile forms in the superficial layers.⁷ The pathogenesis of periodontal tissue commonly thought as a response to bacterial challenge. Infection of periodontal tissues with these and other organisms is accompanied by the release of bacterial endotoxins such as leucotoxins, collagenases, fibrinolysins, and other proteases. Although bacteria are necessary for periodontal disease to take place, a susceptible host is also needed. The host response is essentially protective. However, either hyporesponsiveness or hyper-responsiveness of certain pathways can result in enhanced tissue destruction. Either the host factors or the activity of proteolytic enzymes from bacterial challenges may result in tissue damage. The continuity of periodontal tissues is maintained by homeostasis between regeneration and apoptosis. Apoptosis plays an important role in this disease severity.⁸

Volatile sulphur compounds (VSCs)

Periodontal diseases, in particular, necrotizing ulcerative gingivitis and severe periodontitis, can give rise to malodor. Pericoronitis, dry socket, other oral infections or ulcers can also cause malodor as can any causes of bleeding in the mouth. The large surface area of the tongue and its papillary structure allows it to retain considerable quantities of food and debris which support a large microbial population, giving rise to malodor predominantly by the generation of VSCs such as hydrogen sulphide (H_2S) and methyl mercaptan (CH_3SH).^{9,10} Gram-negative anaerobes are probably the main organisms capable of producing sulphur compounds which they release by putrefaction of oral debris, blood and serum products.⁸ Periodontal organisms including *Treponema denticola*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus* and *Fusobacteria* can produce both sulphides and mercaptans, the main VSCs.⁹ VSCs are found in the gingival crevice and are released particularly from deep periodontal pockets and where there is attachment loss.¹² VSCs are likely to arise mainly from the breakdown of cysteine, cystine, and methionine, peptides and amino acids found free in gingival crevicular fluid, in saliva or produced as a result of proteolysis of protein substrates.¹³ Mass spectrometric (MS) and gas chromatographic (GC) analyses have identified H_2S , methyl mercaptan, dimethyl sulphide, $(CH_3)_2S$, and dimethyl disulphide, $(CH_3)_2S_2$, as the principal malodorous products of salivary putrefaction.^{14–16} Hydrogen sulfide has been shown previously to exert proapoptotic activity. H_2S may cause micronuclei formation (indicating DNA damage) and cell cycle arrest (G_1 phase). It may also resulted in stabilization of p53 coupled with induction of downstream proteins such as p21, Bax, and cytochrome *c*, as well as translocation of Bax from the cytosol to the mitochondria and release of cytochrome *c* from mitochondria. It was

shown that H₂S did not up-regulate cell levels of the antiapoptotic protein, Bcl-2. Mounting evidence indicates that, on DNA damage, p53 promotes apoptosis by activating Bax, which enhances apoptosis by stimulating the release of cytochrome *c* and the formation of apoptosomes. However, the precise molecular mechanisms involved in H₂S-induced apoptosis are, as yet, unknown. It is likely that DNA damage caused by H₂S induces p53, which then activates Bax. Induction of Bax might then promote apoptosis by targeting the mitochondria and releasing cytochrome *c* to cytosol. H₂S causes DNA lesions and up-regulates the genome guardian p53, which leads, in turn, to translocation of proapoptotic Bax and the release of cytochrome *c* and ultimately results in apoptotic cell death.¹⁷

H₂S were reported to induce cell apoptosis and to suppress expression of some leukocyte and endothelial adhesion molecules. The proapoptotic effect of H₂S in cells via activation of mitogen-activated protein kinase pathways has been suggested to be an important endogenous modulator of cellular apoptosis. Several recent reports provide evidence suggesting a role for H₂S in inflammation. H₂S can scavenge peroxynitrite and can interfere with the ability of neutrophils, through hypochlorous acid, to kill microbes and other cells (figure 1). H₂S inhibited the opening of mitochondrial mPTP. The opening of the mPTP plays a pivotal role in the induction of apoptosis in cardiomyocytes. H₂S-mediated inhibition of the mPTP is regulated via the phosphorylation of GSK-3β (Ser9)

(figure 2). The inhibitory effects of H₂S on mPTP opening are mediated by signaling elements such as GSK-3β, and H₂S does not directly act on mitochondrial mPTPs.^{16–20}

Acute exposure to H₂S was also associated with increased expression of toll-like receptor 4 (TLR-4),²⁰ that activate intracellular signaling, resulting in the induction of a variety of effector genes and the production of inflammatory cytokines. This response was coupled with an increase in expression of genes involved in matrix remodeling (e.g., laminin gamma 2, microtubule-associated protein 6, fibrinogen-like 2).

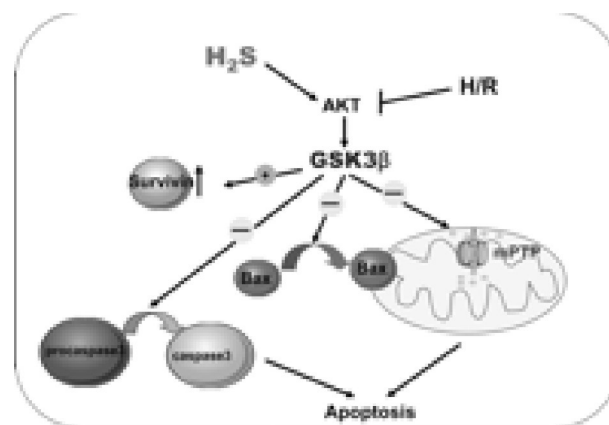


Figure 2. H₂S-mediated inhibition of the mPTP is regulated via the phosphorylation of GSK-3β.²²

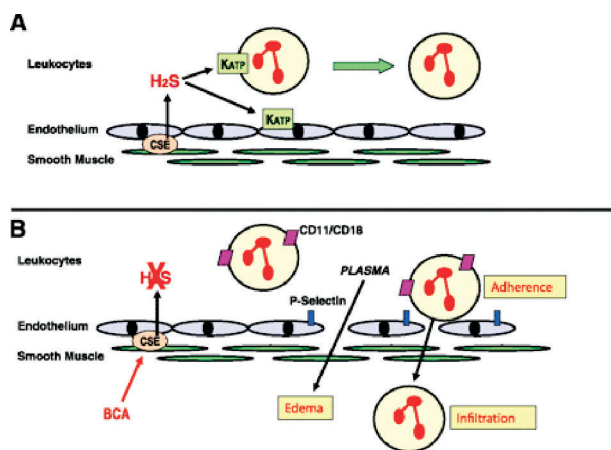


Figure 1. Hydrogen sulfide modulates inflammatory processes at the leukocyte-endothelial interface. *A*) Under normal conditions, H₂S is synthesized in blood vessels primarily via cystathionine-γ-lyase (CSE), which is expressed in endothelial cells and smooth muscle cells. H₂S tonically down-regulates leukocyte adherence via activation of ATP-activated potassium channels (K_{ATP}) on leukocytes and the endothelium. *B*) When endogenous H₂S synthesis is inhibited, leukocyte rolling and adherence to the vascular endothelium increase, likely due in part to elevated expression of adhesion molecules on leukocytes (CD11/CD18) and endothelial cells (P-selectin).²⁰

DISCUSSION

Gingival epithelial tissues play a key role in periodontal pathogenesis by forming a barrier against penetration by periodontally pathogenic microorganisms and the detrimental products of microorganisms. In healthy gingiva, oral epithelium plays a key role as a barrier against pathogens or toxic compounds.²⁰ The maintenance of this epithelial barrier is therefore extremely important for the preservation of normal gingival structure and function. However, during the progression of periodontal disease, this barrier can be affected. Several studies have focused on the relationship between oral malodorous compounds and this epithelial barrier.

VSCs have been reported to increase the permeability of the tissue and the penetration of lipopolysaccharide and prostaglandin in a crevicular epithelial model. A study used a porcine model for gingival crevicular epithelia to show that exposure to concentrations of VSCs much lower than those found in periodontal pockets caused increased permeability of sublingual nonkeratinized mucosa. Another study demonstrated that protein content of CH₃SH-exposed epithelial cell cultures was decreased by approximately 25% and seemed to be irreversible although it was incubated for next-24 hours in a mercaptan-free environment. Furthermore, VSCs induced an important decrease in

the collagen content of the VSC-exposed cell cultures. Another study also demonstrated the capability of VSCs in suppressing collagen synthesis by 39% and increasing the intracellular degradation of newly synthesized collagen from 26% to 42%. Human gingival fibroblasts were exposed to hydrogen sulfide and methyl mercaptan, total protein synthesis was reduced by 18% and 35%, respectively.^{21–23} It seems that increased concentrations of CH₃SH have an inhibitory effect on both cell growth and proliferation in human oral epithelial cell lines. The changes in total protein were accompanied by a corresponding decrease in collagenous protein, which resulted from increased degradation and suppressed synthesis. Methyl mercaptan suppressed DNA synthesis by 44% and altered collagen metabolism in fibroblast cultures. Methyl mercaptan reduces collagen synthesis by 39%, while increases intracellular degradation of newly synthesized collagen by 62%. Hydrogen sulfide was also shown to induce cell cycle arrest in oral epithelial cells by the expression of p21(Cip1) in Ca9-22 cells, which may contribute to delayed epithelial repair. Exposure to 5 and 10 ng/mL of H₂S significantly decreased DNA synthesis. Cell cycle analysis also showed that exposure to both concentrations of H₂S significantly increased the proportion of cells in G₁ phase and significantly decreased the proportion of cells in S phase.²⁴ Volatile sulfur compounds were also reported to increase the production of interleukin-1 and prostaglandin E₂ (PGE₂), activate matrix metalloproteinase, then increase collagen degradation and reduce collagen synthesis in human gingival fibroblasts.²⁵ VSC may also induce osteoclasts activation which may deleterious towards alveolar bone and periodontium. In the murine cell culture, Li et al demonstrated the capability of H₂S to induce pathologic changes in rat alveolar bone. Cathepsin K protein, a specific marker for osteoclasts, was expressed in the H₂S-induced multinuclear cells. Apoptosis plays an important role in the onset and progress of periodontal conditions. Activation of caspase-3, p53 or Bcl-2 was found in human gingival tissues with periodontitis, and periodontal pathogenic microorganisms have been reported to cause apoptosis in periodontal tissues. The initial apical migration of junctional epithelium in lipopolysaccharide-induced experimental periodontitis appeared to occur simultaneously with the apoptosis of periodontal ligament fibroblasts.^{25–27} It was therefore suggested that the apoptosis-related detachment of connective tissue may cause the migration of junctional epithelium. Callenic and others found there were two main mechanisms in the apoptotic process involve activation of an intrinsic pathway, in which the mitochondrion plays a central role, and activation of an extrinsic pathway, involving a receptor–ligand-mediated mechanism.²⁸ To distinguish between the two pathways, they analyzed mitochondrial changes. Increased production of ROS in mitochondria causes disruption of the electrochemical gradient across the inner mitochondrial membrane, which then activates

the apoptotic process.²⁹ They also found that H₂S increased ROS and caused a significant loss of the mitochondrial inner transmembrane potential. Collapse of this potential is associated with early stages of apoptosis; moreover, it leads to a key event in the mitochondrial pathway of apoptosis, that is, the release of cytochrome C from the mitochondria intermembrane into cytosol. In response to H₂S, the release of cytochrome C was significantly increased especially in 48 hours.^{19,30}

H₂S was shown to induce genomic DNA damage in human gingival fibroblasts.^{30,31} Callenic and others also observed an increment in the number of DNA strand breaks at the genomic level, proving the genotoxic effect of H₂S by using single-cell electrophoresis gel.²⁵ Genomic DNA damage suggests that other molecular pathways, such as the p53 pathway, might be involved in the apoptotic process and that H₂S may have pathological effects on human gingiva at the genomic level.

It is concluded that the VSCs, the main source of oral malodor may be considered periodontally-toxic. The VSCs may increase epithelial permeability, trigger the pro-inflammatory cytokines, and induce apoptotic process in periodontal ligament. These may lead to periodontal destruction in susceptible host. It is suggested the VSCs elimination as an integral part of periodontal treatment in any phase. The use of potent VSCs eliminator, such as chlorine dioxide shall be considered. The use of chlorine dioxide and topical antioxidant may be beneficial especially in preliminary and maintenance phase to control the disease progression, and in surgical phase to reduce the deleterious effect of VSCs towards the periodontal regeneration.

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