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Review

Hydrogen sulfide metabolism and its role in the development of periodontal diseases

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Abstract

Hydrogen sulfide (H_2S) is a signaling molecule that is actively synthesized in the tissues and regulates a wide range of physiological processes, namely cardiovascular, neuronal, immune, respiratory, gastrointestinal, liver, and endocrine systems, by influencing cellular signaling pathways and sulfhydration of target proteins. The influence of H_2S signaling in cell cycle and cell death pathways is associated with tumor growth, angiogenesis, and neurodegenerative diseases. The relationship between H_2S and oral cavity pathologies, especially periodontal diseases, is controversial, but should not be underestimated. Further research is needed in order to clarify the exact mechanisms and conditions, which cause the H_2S molecule to exhibit cytoprotective/antioxidant or cytotoxic proprieties in the oral cavity.

Keywords: hydrogen sulfide, metabolism, oral cavity, periodontitis

Hydrogen sulfide (H_2S) is well-known as a toxic gas with an obnoxious odor, which is mainly formed in the process of putrefaction of animal and plant proteins [1]. As a novel endogenous gas transmitter, which mediates a variety of biological processes through multiple signaling pathways [2], H_2S was discovered in 1996, when its formation in brain tissue by enzyme cystathionine-β-synthase (CBS, EC 4.2.1.22) was described and its possible role as a neuromodulator was suggested (promoting the induction of long-term potentiation in the hippocampus of rats due to increased activity of N-methyl D-aspartate receptors) [3]. Today it is known that H_2S regulates a wide range of physiological processes, namely cardiovascular, neuronal, immune, respiratory, gastrointestinal, liver, and endocrine systems, by influencing cellular signaling pathways and sulfhydration of target proteins. The influence of H_2S signaling in cell cycle and cell death pathways is associated with tumor growth, angiogenesis, and neurodegenerative diseases [4].

Endogenous H_2S in humans and animals is synthesized from sulfur-containing amino acids, primarily from L-cysteine and its disulfide form – cystine. Since cysteine can be synthesized in the body from methionine, the synthesis of H_2S can also start with methionine [5]. Enzymes CBS, cystathionine-γ-lyase (CSE, EC 4.4.1.1), 3-mercaptopyruvate sulfurtransferase (3-MST, EC 2.8.1.2)

and cysteine aminotransferase (CAT) are involved in the synthesis of hydrogen sulfide [6]. In the process of the enzymatic pathways, CSE and CBS are cytosolic pyridoxal-dependent enzymes, which lead to the formation of hydrogen sulfide using L-cysteine and homocysteine (Hcy) as the main substrates, while 3-MST uses 3-mercaptopyruvate (3-MP) as the substrate. 3-MP is generated by CAT from L-cysteine and α-ketoglutarate in the presence of cofactors – thioredoxin and dihydrolipoic acid [7–9]. Most of the 3-MST is localized in mitochondria because the concentration of L-cysteine in mitochondria is 3 times higher than in the cytoplasm [7, 10].

D-cysteine can also be a substrate for H_2S synthesis. The synthesis takes place with the help of an enzyme D-amino acid oxidase (DAAO, EC 1.4.3.3) with 3-MST, mainly in the kidneys and cerebellum and is 80 times more productive than the synthesis of H_2S from L-cysteine [10, 11]. H_2S can also be synthesized by thiosulfate-anion reduction using thiosulfate-dithiol sulfur transferase (TST, EC 2.8.1.5) [12].

H_2S plays the role of a signaling molecule, the gas transmitter in the human organisms, however, no specific receptors have been found for it. Different ion channels, receptors, enzymes, and proteins, regulating numerous biochemical and physiological processes, serve as H_2S molecular targets. A key mechanism of H_2S-signaling is S-sulfhydration of proteins, post-translation modification with conversion of -SH groups into -SSH, which significantly increases the reactivity of cysteine residues and increases the functional activity of molecular targets as well [1].

The cytoprotective properties of H_2S probably relate to its ability to neutralize various active forms of molecules. Hydrogen sulfide is known as a free radical scavenger that can directly react and "quench" superoxide anion, peroxynitrite and other reactive oxygen species. The significance of the reaction of H_2S with oxygen is not unambiguous, because the reaction product – sulfite can have both toxic and antioxidant properties, which apparently depends on its concentration [13]. Moreover, hydrogen sulfide has the property of protecting cells from oxidative stress due to its ability to increase the level of intracellular glutathione. The release of H_2S into the extracellular space causes the reduction of cystine to cysteine, which increases the amount of cysteine available as a substrate for glutathione synthesis, and enhances the activity of cystine/glutamate antiporter, thereby increasing the transport of cysteine into cells [10, 14, 15].

In addition, the cytoprotective effect of hydrogen sulfide is associated with the modulation of the function of intracellular caspases or kinases, activation of nuclear factor – NF-kβ and κB-dependent proteins (inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), intercellular adhesive molecule-1), and with a decrease in anti-apoptotic factor Bcl-2 [16–19].

M. Greabu and co-authors (2016) noted that H_2S, on one hand, at low concentrations has antioxidant and cytoprotective effects, while on the other hand – at higher concentrations is cytotoxic [20], causing the activation of free radical processes, calcium mobilization, depletion of the glutathione system, the intracellular release of iron, as well as induction of mitochondrial cell death [16]. Therefore, the rate of utilization of hydrogen sulfide is important. Three different mechanisms are involved in H_2S catabolism: oxidation, methylation, and metalloprotein uptake. The vast majority of hydrogen sulfide is excreted by the kidneys in the form of sulfate [21, 22].

The role of endogenous H_2S produced in the oral cavity is poorly understood. There are data that H_2S is generated by the products of sulfate-reducing bacteria (SRB) metabolism, decomposing substrates such as cysteine, arginine or tryptophan [23]. SRB firstly isolated from the oral cavity are gram-negative mesophilic bacteria belonging to the class Deltaproteobacteria of the phylum Proteobacteria, including 2 orders, such as Desulfovibrionales and Desulfobacterales, of particular interest [24]. In addition, human periodontal stem cells have been shown to express H_2S-synthesizing enzymes – CSE and CBS. Herewith CBS is probably the main source of endogenous H_2S in the periodontium [25].

Chun-Mei and co-authors (2017) collected gingival tissues from patients undergoing periodontal operation including gingivitis, moderate chronic periodontitis, severe chronic periodontitis, and normal controls. RT-PCR and western blotting were performed to measure mRNA and

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protein levels of CBS and CSE, responsible for H_2S production. The mRNA and protein of CBS and CSE were both expressed in human gingiva and raised significantly during moderate and severe periodontitis compared to healthy control. CBS, but not CSE, significantly increased in gingivitis. However, there was no significant difference of H_2S level and synthesis among these groups [26].

Liao and Hua (2013) found that H_2S increases the expression of osteoprotegerin and receptor activator of NF-κB ligand (RANKL) by human periodontal ligament cells, stimulated by the force of tension. Probably, this indicates the involvement of H_2S in the process of periodontal remodeling, especially in the case of teeth displacement [28, 29].

Kushkevych and co-authors (2020) noted the toxic effects of H_2S on the epithelial cells of the oral cavity. H_2S can act as an inhibitor of cellular cytochrome oxidase, and can also have a secondary effect, destroying disulfide bonds of proteins, affecting granulocytes and their function in the immune system [24].

H_2S and other volatile sulfur compounds (VSCs) have been shown to cause halitosis, which means bad breath (synonyms: foeter oris or foeter ex oris) [26], and the protective system of lactoperoxidase of the oral cavity inhibits the growth of microorganisms and the formation of VSCs [29]. Also, VSCs, such as H_2S, mercaptan. and thioether, stimulate the formation of pro-inflammatory cytokines and promote the development of inflammation in endodontic dental treatment [30].

Some studies suggest that H_2S is directly related to the initiation and progression of periodontal disease by inhibiting the proliferation of oral keratinocytes [31], decreasing protein synthesis in oral fibroblasts and inhibiting collagen synthesis [32]. Moreover, H_2S production is associated with many bacterial species that are involved in periodontitis pathogenesis, such as Porphyromonas gingivalis, Treponema denticola, and Fusobacterium spp. Since the higher levels of H_2S have been detected at diseased sites, H_2S has been suggested to serve as a marker for the proteolytic activity of the biofilm and also to be involved in the pathogenesis of the periodontal disease [33].

Greabu and co-authors (2016) also suggest Fusobacterium spp., Parvimonasmicra, Tannerella forsythia or Filifactoralocis being as H_2S-producing bacterial strains [20]. In particular, Fusobacterium spp. acts on substrates such as cysteine [34], Hcy [35], or reduced glutathione [36].

Chu and co-authors (2002) studied the metabolism of reduced glutathione in Treponema denticola [37]. Researchers have proposed three stages of bacterial H_2S production: a) glutamate or glutamine and the dipeptide cysteinyl glycine (Cys-Gly) are formed as a result of transformations of reduced glutathione; b) glycine and L-cysteine are formed as a result of Cys-Gly breakdown; c) pyruvate, ammonia and H_2S are the final products of L-cysteine degradation.

In vitro studies have shown that treatment of cultures of Fusobacterium nucleatum and Porphyromonas endodontalis with the stress hormones adrenaline, noradrenaline and cortisol inhibited their growth, but increased their H_2S production. At the same time, stress hormones did not affect the growth of Prevotela intermedia and Porphyromonas gingivalis, but increased the generation of H_2S by Prevotela intermedia [38].

Chi and co-authors (2014) also found that the periodontal pathogen Porphyromonas gingivalis produces H_2S [39]. Herewith, the level of H_2S in the oral cavity was positively correlated with the index of bleeding gums, the depth of periodontal pockets, and radiographic data on bone loss. To elucidate the mechanism of this correlation, gum fibroblasts and periodontal ligament cells (alveolar periosteum) were treated with different concentrations of H_2S donor – sodium hydrosulfide (NaHS) in the presence or absence of Porphyromonas gingivalis. H_2S not only increased dose- and time-dependent expression of mRNA and proteins of pro-inflammatory cytokines IL-6 and IL-8 in gum fibroblasts and periodontal ligament cells, but also increased the expression of these cytokines, which was caused by the lipopolysaccharide of Porphyromonas gingivalis.

Interestingly, for the experimental treatment of periodontitis in rats, another group of researchers administered NaHS in three different doses (14, 28, and 70 μmol/kg), but did not get a

positive effect (loss of alveolar bone did not differ from that in untreated animals) [40].

There are data that H_2S can increase the permeability of the gum epithelium and induce apoptosis of cells in the periodontium, including epithelial cells and gum fibroblasts, periodontal ligaments, and osteoblasts [31, 41–43]. It was found that in most of the studied cell types: oral fibroblasts, oral keratinocytes, oral keratinocyte stem cells and total keratinocyte stem cells, apoptosis happened by the internal – mitochondrial pathway. The external pathway of apoptosis (ligand-mediated) happened only in osteoblasts and cells isolated from the alveolar bone.

In contrast to studies suggesting that H_2S is directly related to the initiation and progression of periodontal disease, Basic and Dahlen (2015) in their research, which included 43 patients with severe or moderate periodontitis, did not establish a significant correlation between the level of H_2S and the severity of periodontitis or the composition of the oral microflora [44]. Moreover, there are data that endogenous H_2S, in contrast, has a periodontal protective effect [38]. Gugliandolo E. and co-authors (2018) compared the protective effects of ketoprofen with ATB-352, a hydrogen sulfide-releasing derivative of ketoprofen, in a lipopolysaccharide (LPS) model of periodontitis in rats. Their results showed that 14 hours after intragingival injection of LPS, there was high tissue damage associated with bone resorption, and in gingivomucosal tissues, there was a significant expression of NF-kb p65 and pro-inflammatory cytokine as well as a higher expression of COX-2 and iNOS, activation of the apoptotic process. Treatment with ATB-352 at the dose of 20 mg/kg, was able to reduce the inflammatory process associated with intragingival LPS injection and also had a positive effect on bone resorption and tissue damage [45].

Altogether, data presented in recent studies suggest that H_2S is a signaling molecule that is actively synthesized in the tissues and regulates a wide range of physiological processes, namely cardiovascular, neuronal, immune, respiratory, gastrointestinal, liver, and endocrine systems, by influencing cellular signaling pathways and sulfhydration of target proteins. The influence of H_2S signaling in cell cycle and cell death pathways is associated with tumor growth, angiogenesis and neurodegenerative diseases. The relationship between H_2S and oral cavity pathologies, especially periodontal diseases, is controversial, but should not be underestimated. Further research is needed in order to clarify the exact mechanisms and conditions, which cause the H_2S molecule to exhibit cytoprotective/antioxidant or cytotoxic proprieties in the oral cavity.

Conflict of Interest

The authors declare no conflict of interest

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