Importance of bacterial hydrogen sulfide in the pathogenesis of periodontal diseases

Akademisk avhandling

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av Amina Basic
leg. Tandläkare

Fakultetsopponent:

Professor Philip Marsh
University of Leeds, UK

Avhandlingen baseras på följande delarbeten

I. Basic A, Blomqvist S, Carlén A, Dahlén G. Estimation of bacterial hydrogen sulfide production *in vitro*. *Journal of Oral Microbiology 7. 2015.*

II. Basic A, Dahlén G. Hydrogen sulfide production from subgingival plaque samples. *Anaerobe 35:21-27. 2015.*

III. Basic A, Blomqvist M, Dahlén G, Svensäter G. The proteins of *Fusobacterium* spp. involved in hydrogen sulfide production from L-cysteine. *BMC Microbiology 17. 2017.*

IV. Basic A, Alizadehgharib S, Dahlén G, Dahlgren U. Hydrogen sulfide exposure induces NLRP3 inflammasome-dependent IL-1β and IL-18 secretion in human mononuclear leukocytes *in vitro*. *Clinical and Experimental Dental Research 3:115-120. 2017*

V. Basic A, Serino G, Leonhardt Å, Dahlén G. Induction of interleukin (IL)-1β and IL-18 secretion by hydrogen sulfide in periodontitis patients and healthy controls: a clinical cross-sectional study. *In manuscript*

SAHLGRENSKA AKADEMIN
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Importance of bacterial hydrogen sulfide in the pathogenesis of periodontal diseases

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Abstract

Hydrogen sulfide (H_2S) is one of many end-products of the proteolytic activities in the subgingival microbiota in patients with periodontal diseases, such as gingivitis and periodontitis. Although H_2S is generally regarded as toxic, the mechanisms that underlie its production and its effects on human cells and tissues are poorly understood. Therefore, the role of H_2S in the pathogenesis of periodontal diseases was investigated. Two colorimetric methods, the bismuth test (BT) and the methylene blue (MB) method, were used to estimate the amounts of H_2S produced by the bacteria *in vitro* and *ex vivo* (**Papers I, II and V**). Oral bacteria, e.g., *Fusobacterium* spp., *Porphyromonas gingivalis* and *Treponema denticola,* were found to have strong capacities to degrade cysteine and produce H_2S *in vitro* (**Paper I)**. The *Fusobacterium* spp. were found to express several enzymes that are involved in the production of H_2S. The expression patterns of the different enzymes varied among *Fusobacterium* subspecies and strains (**Paper III**). In an *ex vivo* experiment using BT, we showed that the subgingival plaques of subjects (N=43) with poor oral hygiene had the capacity to produce H_2S **(Paper II).** High levels of periodontitis-associated bacteria were detected, and the BT values reflected the proteolytic activities of the bacteria and gingival inflammation rather than disease progression and periodontitis. A correlation between a positive BT and gingival inflammation was confirmed in **Paper V**, where H_2S-producing bacteria were significantly more prevalent in the subgingival pockets of periodontitis patients (N=32) than of healthy controls (N=32), which indicates potent bacterial proteolytic activities in the untreated deep periodontal pockets. **Paper IV** described how the peripheral blood mononuclear cells (PBMCs) of blood donors and a monocytic cell line increased their secretion of the pro-inflammatory cytokines IL-1β and IL-18 *in vitro* when exposed to the H_2S-donor sodium hydrosulfide (NaHS). This secretion was shown to be mediated by the NLRP3 inflammasome. These results were verified in **Paper V**, where the PBMCs of periodontitis patients and healthy controls secreted significantly higher levels of IL-1β and IL-18 when exposed to NaHS. In addition, both unexposed and exposed PBMCs of the periodontitis patients secreted higher levels of the two cytokines than the corresponding cells of healthy controls. These results suggest that the susceptibility of the host to develop disease can be attributed in part to enhanced secretion of pro-inflammatory cytokines following exposure to bacterial metabolites, such as H_2S. In summary, toxic bacterial metabolites, such as H_2S, may play an important role by affecting the cells of the host immune system, thereby inducing and sustaining gingival inflammation.

Keywords: Hydrogen sulfide, Periodontitis, Gingivitis, IL-1β, IL-18, NLRP3 inflammasome, Oral microbiota, *Fusobacterium* spp., Bismuth test, L-cysteine

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