Journal of
PERIODONTAL RESEARCH

J Periodont Res 2010; 45: 71–78

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2009.01202.x

Hydrogen sulfide induces apoptosis in human periodontium cells

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Zhang J-H, Dong Z, Chu L. Hydrogen sulfide induces apoptosis in human periodontium cells. J Periodont Res 2010; 45: 71–78. © 2009 John Wiley & Sons A/S

Background and Objective: The existence of hydrogen sulfide (H_2S) at high concentrations in periodontal pockets is a characteristic feature of periodontitis. Periodontal pathogens play a key role in the production of H_2S under these etiology conditions. This study was designed to examine the cytotoxicity of H_2S in periodontium cells, including human periodontal ligament (PDL) cells and human gingival fibroblasts (HGFs), as well as the role of H_2S in apoptosis induction.

Material and Methods: Human PDL cells and HGFs were cultured in the presence of Na_2S/HCl or in the presence of H_2S produced enzymatically by the action of *Treponema denticola* cystalysin (L-cysteine desulfhydrase) on L-cysteine. Apoptosis was assessed morphologically after nuclear staining with DAPI or was quantified by flow cytometry after staining with annexin V. Caspase activation was measured by an enzymatic assay using DEVD-AMC, a synthetic caspase substrate.

Results: Among the three products obtained following degradation of L-cysteine by *T. denticola* cystalysin, only H_2S induced significant apoptosis in HGF cells. Hydrogen sulfide also induced typical apoptotic morphology in cultured PDL cells. The changes were dependent on the H_2S dose and on the treatment time with H_2S. Hydrogen sulfide-induced apoptosis was also confirmed by staining with annexin V and propidium iodide. In addition, treatment with H_2S led to caspase activation in these cells.

Conclusion: These results showed that physiological concentrations of H_2S can induce apoptosis of PDL cells and HGFs in periodontitis, suggesting that H_2S may play an important role in periodontal tissue damage in periodontal diseases.

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Key words: hydrogen sulfide; apoptosis; periodontium cells; *Treponema denticola*; cystalysin

Accepted for publication December 16, 2008

The existence of high concentrations (up to and greater than 2 mM) of volatile sulfur compounds in periodontal pockets is an important characteristic of periodontal diseases (1,2). Hydrogen sulfide (H_2S), which has been identified as a malodorous and highly toxic factor (3), is a major and well-characterized component in this group of volatile sulfur compounds (1,2). Previous studies have demonstrated that oral bacteria play key roles in the production of the sulfide compounds in sites with periodontal disease (4,5).

Both local infections and peripheral multiple infections play a significant role in the initiation and development of human periodontal diseases (6,7). *In vitro*, several putative periodontal pathogens have been reported to induce cytotoxicity of periodontium cells (8). However, the pathologic processes of periodontal diseases have not been fully determined. Recently, apoptotic cell death was found to play an important role in various oral diseases (9). Based on clinical pathological analyses of gingival tissue biopsies from adults with chronic periodontitis and from healthy controls, Gamonal *et al.* (10) reported that apoptosis was induced in human periodontal tissue by host and microbial factors and they developed the hypothesis of apoptotic mechanisms being important in the inflammatory process associated with gingival tissue destruction observed in adults with periodontitis. Several

reports indicated that some periodontal pathogens, their lipopolysaccharides (LPSs) and some potential virulence factors might induce apoptosis in different cells (11–13). However, as a common toxic factor, the cytotoxicity of H_2S on periodontium cells has not yet been fully explained.

Several previous studies suggested that H_2S is a toxic factor in human clinical cases and in animal experiments (3). However, the mechanism of cell damage that occurs during treatment with H_2S is unclear. Recently, H_2S was demonstrated to induce apoptosis in insulin-secreting beta cells by enhancing endoplasmic reticulum stress via p38 MAPK activation (14). In 2006, the overexpression of cystathionine γ-lyase was found to stimulate apoptosis in human aorta smooth muscle cells through the increased endogenous production of H_2S (15). In 2007, in an experiment carried out to elucidate the mechanism(s) by which H_2S affects cell growth and function, H_2S was demonstrated to be capable of inducing DNA damage and changes in apoptotic gene expression in human lung fibroblast cells (16). More recently, it has been reported that a mixture flow of H_2S and air can induce apoptosis of human gingival fibroblasts (HGFs) (17). However, high concentrations of H_2S (2) and low levels of oxygen are characteristic features in periodontal pockets with periodontitis (18). The influence of these unique conditions on cells of the periodontium is less well understood.

While high concentrations of H_2S are consistently produced and maintained in sites of periodontal disease, only a few enzymes have been identified to produce H_2S from L-cysteine and other substrates. We have previously examined a three-step pathway for glutathione catabolism that results in the production of H_2S by *Treponema denticola* (19). In this pathway, γ-glutamyltransferase, cysteinylglycinase and cystalysin sequentially hydrolyze glutathione to the final products, ammonia, pyruvate and H_2S (20–22). However, whether all of these enzymatic products damage cells, and how H_2S affects cells of the periodontium, is still unclear.

In this study, we used recombinant *T. denticola* cystalysin to determine the effects of L-cysteine degradation products on cells of the periodontium. The results demonstrated that of the three L-cysteine degradation products (ammonia, pyruvate and H_2S), only H_2S induced significant apoptosis in HGFs. The addition of Na_2S/HCl also resulted in typical apoptosis in human primary periodontal ligament (PDL) cells. Moreover, H_2S may induce ligament cell apoptosis via caspase activation.

Material and methods

Materials used and preparation of enzymes from *T. denticola*

Recombinant *T. denticola* cystalysin was purified from *Escherichia coli* cells using the appropriate expression clones, as previously described (20). Unless otherwise indicated, all chemicals and reagents were obtained from Sigma Chemical Company (St Louis, MO, USA).

Cell culture and kinetic analysis of H_2S production and maintenance

Cell culture — Human PDL cells and HGFs were kind gifts of Dr David Cochran's laboratory (University of Texas Health Science Center, San Antonio, TX, USA) (23,24). Both cell types were cultured in Dulbecco's modified Eagle's minimal essential medium (Invitrogen, Carlsbad, CA, USA), containing 10% fetal bovine serum, at 37°C in an atmosphere of 5% of CO_2. These cells were used between passages 5 and 10.

Kinetic analysis of H_2S production and maintenance — To identify suitable experimental conditions for analyzing the kinetics of production of H_2S, ammonia and pyruvate, purified cystalysin from *T. denticola* was added, together with L-cysteine, to the cell culture medium at final concentrations of 1 μg/mL and 2 mM, respectively, in three different volumes of culture (70, 35 and 20 mL). To determine changes in H_2S concentrations following the generation of chemically produced H_2S, Na_2S was neutralized, using an equivalent molarity of HCl, to pH 7.4 in phosphate-buffered saline (PBS) and the mixture was immediately added to the culture medium. The conditions used are described later (under the heading 'Cell treatments'). After incubation for 0, 20 or 40 min, or for 1, 2, 3, 4, 24, 36, 48, 72, or 96 h, 0.1-mL samples were collected from the culture to determine the concentration of H_2S. Incubation medium harvested from cell cultures was centrifuged and supernatant fluids were collected as samples. The concentration of H_2S was determined as described by Siegel (25), with some modifications (20). The sulfide concentration was determined from an Na_2S standard curve. The concentrations of ammonia and pyruvate were determined as previously described (20).

Analysis of oxygen reduction following the addition of H_2S — The concentration of oxygen was also determined as previously described (26). Briefly, an SM 600 dissolved oxygen meter (Milwaukee Instruments Inc. Menomonee Falls, WI, USA) was used to determine the concentration of oxygen, according to the manufacturer's instructions. The electrode was calibrated to zero before every measurement using the special solution provided by the manufacturer, and the concentration of oxygen in each sample was measured at room temperature. To measure the oxygen concentration of the medium in 70-mL culture flasks, the flask was gently shaken, then 1.5 mL of the culture medium was taken from the center of the flask, using a pipette, and transferred into a small cup. The SM 600 electrode was gently shaken in the culture medium of the cup to permit a a stable reading of the oxygen concentration to be obtained. All analyses were carried out in triplicate unless otherwise indicated.

Cell treatments

Human PDL cells or HGFs were cultured in T25 flasks (cat. no. 430168, Plug Seal Cap; Corning Incorporated, Corning, NY, USA) until subconfluent. For morphological experiments, 1 × 0.5 cm coverslips were laid on the bottom of each flask before the cells were seeded. When the cells were treated, the flasks were first filled with

70 mL of the culture medium. The test reagents were then added and the flasks were capped immediately using a sealed cap.

Effects of T. denticola cystalysin and its substrate L-cysteine on HGFs (23) — Equal volumes of 50 mM L-cysteine, HCl and 50 mM NaOH were mixed, added (to a final concentration of 2 mM in the culture flask) with cystalysin at 1 μg/mL (final concentration) into the above flask and then sealed immediately with a cap. The cells were cultured at 37°C for the indicated periods of time. In order to determine the effects of the enzyme products separately from the reaction of cystalysin and L-cysteine, 2 mM Na_2S/HCl or ammonia or pyruvate (final concentrations), after neutralization with the equivalent molarity of HCl, were added into the experimental cultures and inoculated under the conditions described above.

Effects of H_2S on PDL cells — Periodontal ligament cells (24) were used to test the effect of H_2S on periodontal cells. Two concentrations (5 and 0.5 mM final concentrations) of Na_2S and HCl were used for treatment of the cells. Following the addition of Na_2S to the culture medium, the same molarity of HCl was added, with mixing, to the flask in which the cells were cultured. The experiments were repeated three times.

Controls for HGF cells and PDL cells — In the first control, HGFs and PDL cells were cultured, as described above, in flasks filled with 70 mL of the medium without any treatment. In the second control, HGF and PDL cells, cultured in flasks filled with 70 mL of medium and incubated in an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, MI, USA) with mixed gases (5% CO_2, 10% H_2 and 85% N_2). These two controls were used to measure the basal levels of cell death in the absence of H_2S.

Nuclear staining

At the indicated time-points, the coverslips with cells were washed twice with PBS and stained for 10 min with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI; Invitrogen) at a concentration of 0.5 μg/mL in PBS. The DAPI solution was removed and the samples were fixed with 4% paraformaldehyde in PBS for 30 min at room temperature and then examined using a fluorescent microscope fitted with a DAPI filter set.

Annexin V/propidium iodide staining and flow cytometry analysis

An annexin V–fluorescein isothiocyanate (FITC) kit (BioVison, Mountain View, CA, USA) was used according to the supplier's protocol. Briefly, cells growing on the bottom of each flask were trypsinized and collected by centrifugation. Approximately 5×10^5 cells were washed twice with PBS and resuspended in 500 μL of 1× binding buffer. Five microlitres of annexin V and 5 μL of propidium iodide were then added to the cell suspensions and the samples were incubated in the dark at room temperature for 5 min. The fluorescence observed following annexin V–FITC (which binds to apoptotic cells) binding and propidium iodide (which stains necrotic cells) staining was measured quantitatively using a flow cytometer.

Analysis of caspase activity

Caspase activity was measured in whole-cell lysates of the samples in an enzymatic assay using the caspase substrate N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (DEVD-AMC) (BIOMOL International LP, Plymouth Meeting, PA, USA) according to the manufacturer's protocol. Briefly, 5×10^6 cells were lysed in 50 μL of cold lysis buffer. Fifty microlitres of 2× reaction buffer and 5 μL of DEVD-AFC (1 mM) were then added and the reaction was carried out at 37°C for 1 h. The intensity of fluorescence was measured with a fluorometer using a 400-nm excitation filter and a 505-nm emission filter.

Results

Kinetics of the production and maintenance of H_2S

We have previously identified the involvement of cystalysin (L-cysteine desulfhydrase) in the degradation of L-cysteine into H_2S, ammonia and pyruvate (20). As H_2S is a volatile thiol compound that can be unstable under some conditions, in this study we first of all developed optimal conditions for the production and maintenance of H_2S from *T. denticola* cystalysin and L-cysteine, or the added chemical compound, in culture medium. The results showed that the addition of cystalysin and L-cysteine resulted in the production of H_2S almost immediately in the culture and that the concentrations of H_2S were highest (for each condition used in this study) after incubation for 2.5 h. The high concentrations of H_2S in the medium remained almost unchanged for at least 30 h and then slowly decreased (Fig. 1A). As H_2S was maintained optimally in flasks containing 70 mL of culture (compared with flasks containing 20 or 35 mL of culture), 70-mL culture volumes were used in all further experiments. In order to determine the kinetics of H_2S production directly in culture medium, changes in the concentration of H_2S were analyzed following the addition of equal molar volumes of Na_2S and HCl. As predicted, the results suggested that the selected conditions were able to maintain high concentrations of volatile H_2S at for at least 30 h, as with the use of cystalysin (Fig. 1B).

The concentrations of ammonia and pyruvate, two other end products occurring from the reaction of the purified cystalysin with L-cysteine, were also determined. As these two products are not volatile, the results suggested that there were no significant changes in their respective levels during the observation period (data not shown).

As predicted, the concentrations of O_2 in the culture medium were significantly decreased by the addition of L-cysteine and cystalysin, or of Na_2S/HCl, as described previously (26). These results indicated that the original concentration of O_2, of approximately 6 mg/L, in the medium was reduced to 0–0.2 mg/L within 2 h and the O_2 levels did not subsequently increase because the flasks were sealed.

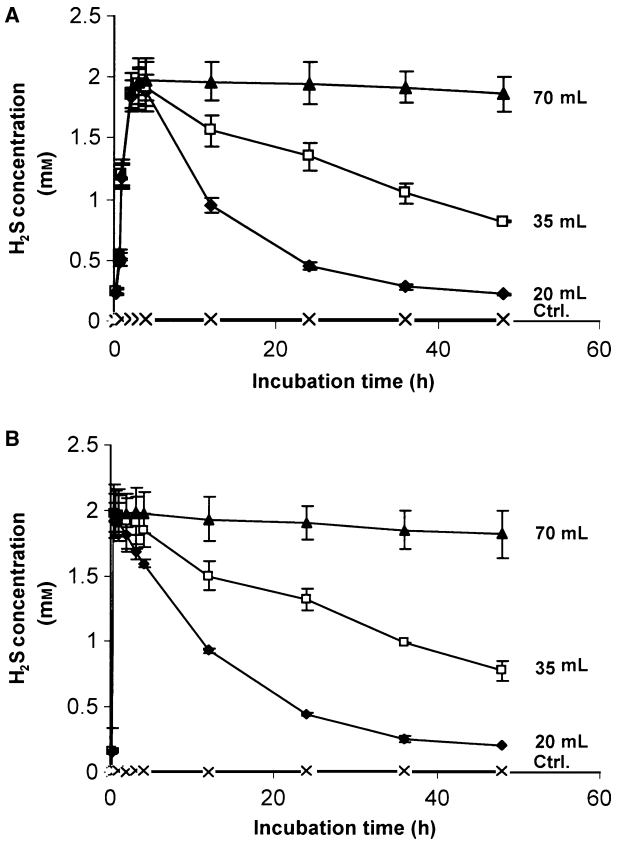

Fig. 1. Kinetic analysis of sulfide levels in culture medium after the addition of *Treponema denticola* cystalysin plus L-cysteine (A) or after the addition of Na_2S/HCl (B). In panel A, a final concentration of 2 mM L-cysteine and 1 μg/mL of *T. denticola* cystalysin were used in all conditions. Four conditions were used in this study: 70, 35 and 20 mL of culture in each flask, and a control of 70 mL of culture medium only. The concentrations of hydrogen sulfide (H_2S) were determined and the results are shown as independent curves. Panel B presents the kinetics of H_2S generated by Na_2S/HCl. The volume used in each experiment was the same as described for (A) and similar results were obtained. Each experiment was carried out three times and error bars indicate standard errors.

H_2S is the apoptosis-inducing end product produced by *T. denticola* cystalysin

Next, we examined the effects of the three end products produced from *T. denticola* cystalysin and its substrate, L-cysteine (20), on periodontal cells. Specifically, we sought to determine if any of the three end products – pyruvate, ammonia, or H_2S – could induce cell death. Human gingival fibroblasts were cultured with the enzyme cystalysin, its substrate L-cysteine, or with each end product, and apoptotic cell death was monitored by nuclear DNA staining, as described earlier. Two of the most important morphological changes distinguishing apoptosis from other types of cell death are chromatin condensation and DNA fragmentation (28,29), both of which can be visualized using DNA-staining dyes such as DAPI or Hoechst 33258 (27). The percentage of cells with apoptotic nuclear morphology is shown in Fig. 2. Compared with control conditions, neither cystalysin nor its substrate, L-cysteine, alone triggered apoptotic cell death, as quantified by chromatin condensation and DNA fragmentation. When both the enzyme and the substrate were added to the culture, at either 2 or 0.5 mM, significant cell death was induced. Among the three enzymatic products, only H_2S was able to trigger cell death; pyruvate and ammonia (NH_3) could not (Fig. 2).

H_2S induces apoptotic morphological changes in cultured PDL cells

In order to expand our observations, another cell type important in periodontal tissue, PDL cells, was used to test, in greater detail, the effect of H_2S on cell damage. We treated PDL cells with 0.5 and 5 mM H_2S and observed nuclear morphology at 24 and 48 h using a fluorescence microscope. At a concentration of 5 mM, H_2S induced dramatic chromatin condensation and nuclear DNA fragmentation changes at both 24 and 48 h. When used at 0.5 mM, H_2S also induced similar dramatic changes at 48 h, but at 24 h the changes were much less. Typical nuclear changes are shown in Fig. 3. Figure 3A shows untreated PDL cells and Fig. 3B,C show PDL cells with apoptotic changes after 48 h of treatment with H_2S. Ligament cell shrinkage and plasma membrane blebbing, two other apoptosis indications, were also shown by phase contrast microscopy (data not shown). These observations provided direct evidence that H_2S induces PDL apoptotic, rather than necrotic, cell death (28,29).

Annexin V binding reveals apoptotic plasma membrane changes in PDL cells treated with H_2S

When cells undergo apoptosis, the plasma membrane loses its asymmetry after insertion of phosphatidylserine in the outer leaflet, which can then bind to annexin V (30). We thus measured binding of FITC–labeled annexin V by using both fluorescence microscopy and flow cytometry (31,32). Evaluation of the number of positively stained cells (Fig. 4), or quantitative measurement by flow cytometry (Fig. 5), revealed that the number of annexin V-positive PDL cells increased after exposure to H_2S for 24 or 48 h, while there were $< 5\%$ positive cells in control cells at the same time-points. The insertion of phosphatidylserine into the plasma membrane is one of the earliest signs of apoptosis (33). We observed substantial levels of annexin V-positive cells as early as 4 h when cells were treated with as little as

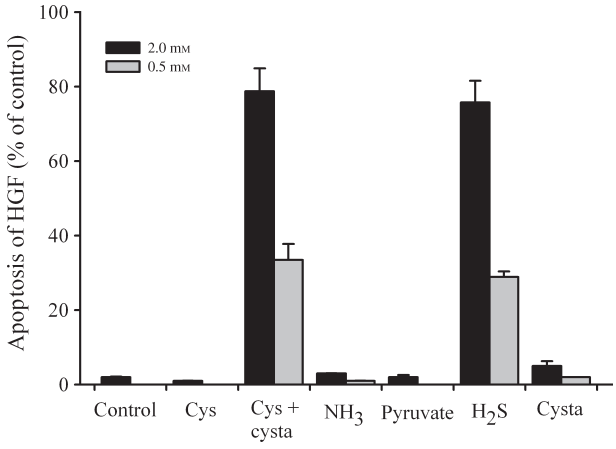

Fig. 2. Effect of cystalysin from *Treponema denticola*, its substrate L-cysteine and enzyme products on apoptosis of human gingival fibroblast. Human gingival fibroblasts were treated with different reagents, and 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) staining was used to evaluate nuclear DNA condensation and chromatin fragmentation. The results are expressed as the percentage of cells with the nuclear morphological change. Each experiment was carried out three times. Error bars indicate standard errors. Cys, L-cysteine; Cysta, cystalysin; H_2S, hydrogen sulfide; NH_3, ammonia.

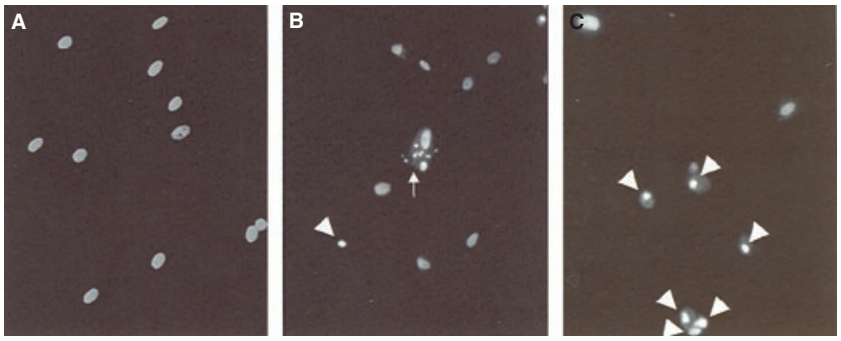

Fig. 3. Hydrogen sulfide (H_2S)-induced periodontal ligament (PDL) cell apoptosis. The PDL cells were treated with 0.5 mM (B) or 5 mM (C) H_2S for 48 h. Nuclear DNA fragmentation (arrow) and chromatin condensation (arrowheads) were revealed by 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) staining and fluorescence microscopy. (A) Control cells at 48 h.

0.5 mM H_2S, in contrast to the control cells, in which positively stained cells were rarely visualized, even at 48 h (Figs 4 & 5). As an additional control, incubation of HGFs or PDL cells in an anaerobic chamber did not induce significant apoptosis. For example, anaerobic incubation of HGFs for 48 h only induced 4–5% of apoptosis, a level comparable to that induced by normoxic incubation (3–4%). Also, similar results for PDL cells were 3–5% and 3–4%, respectively. These results suggested that apoptosis occurring after incubation of cells with H_2S in a sealed flask was mainly a result of the toxicity of H_2S and of the lack of oxygen.

H_2S induces caspase activation in cultured PDL cells

To delineate in greater detail the mechanism of apoptosis induced by H_2S, we examined caspase activation, one of the biochemical hallmarks of apoptosis (34–36). We assayed caspase activity using a fluorogenic peptide substrate (37). For PDL cells, 24 h of treatment with H_2S dramatically induced caspase activity, with increases of more than twofold (0.5 mM) or fourfold (5 mM) observed ($p < 0.05$, respectively) (Fig. 6).

Discussion

In this study, H_2S, generated either chemically or from *T. denticola* cystalysin-catalyzed L-cysteine degradation, was investigated for its cytotoxic effects on human HGFs and PDL cells. The results demonstrated that these cells showed typical cellular and nuclear morphological changes of apoptosis after the treatment with H_2S. Staining with DAPI and annexin V further demonstrated that typical, significant apoptosis took place after treatment with H_2S and that the induction of apoptosis with H_2S was dose-dependent and time-dependent. In addition, the results suggested that caspase activation is involved in the H_2S-induced apoptosis of PDL cells.

High concentrations of H_2S and low levels of oxygen are characteristic features in sites of periodontal disease (1,18). As H_2S is a volatile compound, it is necessary to generate suitable experimental conditions for using to evaluate the actual effects of the volatile gas on cell damage. In this regard, Yaegaki *et al.* successfully developed a design for an H_2S incubation system (17). In this system, both H_2S and non-H_2S (air) chambers were infused with a constant flow of prewarmed and humidified 5% CO_2 in air. These conditions are suitable for studying oral malodorous compounds, although they require special equipment. To evaluate the specific conditions of high concentrations of H_2S and low levels of O_2 in periodontal pockets, important features of periodontitis (1,2,18), as well as H_2S as a volatile sulfur gas, in this study we developed a system to test the influence of H_2S generated either chemically of from enzyme products. Our results suggested that 70 mL of culture medium in flasks is suitable for maintaining high concentrations of H_2S and low concentrations of oxygen, as found in periodontal pockets (1,18). Under these conditions, the observation of H_2S-induced apoptosis of HGFs and PDL cells may be relevant to the conditions in periodontal pockets. Although anaerobic

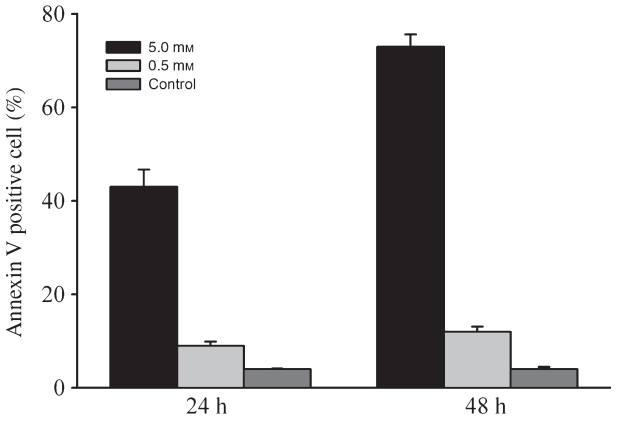

Fig. 4. Hydrogen sulfide (H_2S)-induced apoptosis in periodontal ligament (PDL) cells. Cultured PDL cells were treated with 5 or 0.5 mM H_2S for 24 or 48 h. Cells were labeled with annexin V–fluorescein isothiocyanate (FITC) conjugate, and positive and negative cells were counted under a fluorescence microscope. A total of 300 cells were counted in each sample. Data shown are the percentage of annexin V-positive cells in each sample. Each experiment was carried out three times and error bars indicate standard errors.

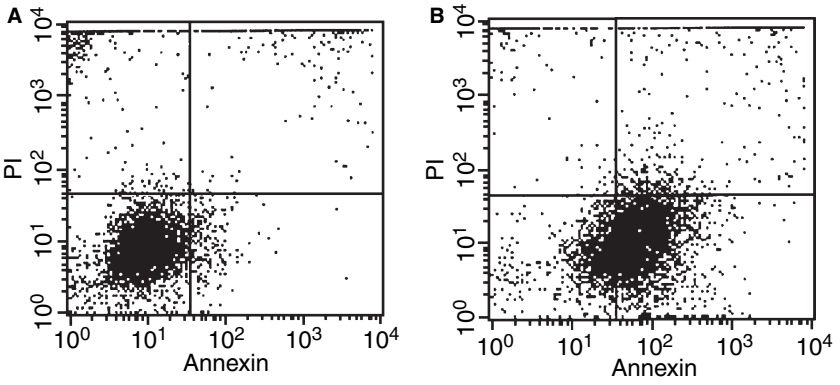

Fig. 5. Annexin V assay of hydrogen sulfide (H_2S)-induced apoptosis in periodontal ligament (PDL) cells. Cultured PDL cells were treated with 0.5 mM H_2S for 48 h. Cells were labeled with annexin V– fluorescein isothiocyanate (FITC) conjugate and quantified following analysis in a fluorescence-activated cell sorter (FACS). (A) control; (B) treatment. PI, propidium iodide.

incubation alone did not induce significant apoptosis, further studies are necessary to determine if the decrease in oxygen concentrations increases the toxicity of H_2S.

Three enzyme end products (ammonia, pyruvate and H_2S) are produced from L-cysteine degradation catalyzed by *T. denticola* cystalysin (19,20). Although some reports have suggested that ammonia and pyruvate are capable of inducing apoptosis in other cell types (38,39), the results presented in this study suggest that these two products do not induce significant apoptosis in HGFs and PDL cells under our experimental conditions. Whether the distinct findings resulted from differences in the host cells utilized, or differences in the doses of the compounds tested (38,39), remain to be clarified by further investigation. However, low (0.5 mM) and high (2–5 mM) concentrations of H_2S that are found in periodontal pockets (2) were able to induce significant apoptosis in our experiments.

While H_2S has been studied as a well-known toxic gas for more than 100 years, remarkable observations have been recently suggested regarding the physiological functions of the gas. The thiol compound may actually work as a physiologically important signaling molecule, as a neurotransmitter and as a neuroprotectant in oxidative stress (40,41), for relaxing vascular smooth muscles by the activation of K_{ATP} channels and for inhibiting smooth muscle cell proliferation via the mitogen-activated protein kinase signaling pathway (42). Other biological functions of H_2S have also been discovered (43,44). Specifically, the observations regarding the regulation of inflammatory responses and anti-inflammatory effects (44) raise an interesting question regarding the toxicity of the compound. Notably, whether H_2S is toxic or has physiological functions probably depends on its concentration (44,45). The physiological concentrations of H_2S in mammalian serum and tissues have been reported to be in the 30–100 μM range, whereas at concentrations above 100 μM, H_2S can exert toxic effects on some organs or tissues (46). In our study, 0.5 mM (500 μM) H_2S showed a significant toxic effect on HGFs and PDL cells. Because this is within the average concentration of H_2S found in periodontal pockets (2), our findings may have relevance to the pathogenesis of periodontal diseases. Another relevant observation is the demonstration of sulfide-detoxifying enzymes in the human colon. Ineffective detoxification may result in mucosal insult, inflammation and ultimately in colorectal cancer (46,47). If this holds true for periodontal tissues, these tissues may also have evolved similar mechanisms to cope with H_2S toxicity.

Several reports suggested that some periodontal pathogenic oral bacteria, including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia*, and some potential virulence factors, might induce apoptosis in different cells (11–13). Among the potential virulence factors, LPS may play an important role in inducing apoptosis of periodontium cells because they are common pathogenic components of bacteria (12,48). Based on some reports, H_2S shares some of the toxic and inflammatory functions of LPS. For example, LPS induces apoptotic cell death and up-regulates the expression of cytokines and other tissue factors (48,49).

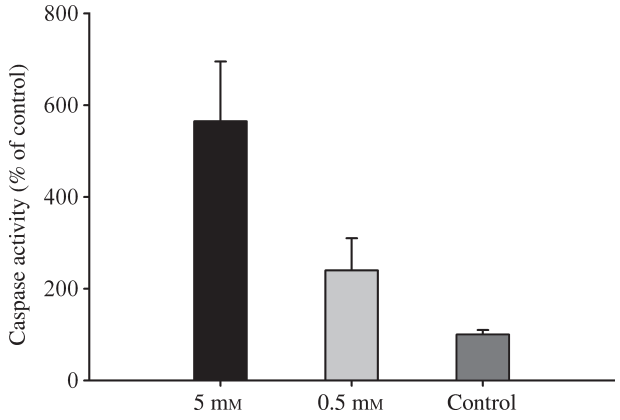

Fig. 6. Hydrogen sulfide (H_2S)-induced caspase activation in periodontal ligament (PDL) cells. Cultured PDL cells were treated with 0.5 or 5 mM H_2S for 24 h. Caspase activity was measured in whole-cell lysates of each sample by an enzymatic assay using the caspase substrate DEVD-AMC. Data shown (three experiments) represent relative enzymatic activity compared with the control samples (mean ± standard deviation).

Periodontal diseases, including periodontitis, are initiated and mediated by inflammation induced by multiple gram-negative bacteria and are characterized by the destruction of the periodontal tissue. Importantly, apoptosis was shown to be the cause of major cell damage in the periodontal tissue in chronic adult periodontitis (10). Recently, investigators also reported that caspases are the key mediators of apoptosis in gingival tissue from patients with periodontitis (50). Together with these studies, our work has provided compelling evidence for the involvement of apoptotic mechanisms in periodontal diseases involving damage of gingival and periodontal tissues. In view of the fact that H_2S is constantly produced in periodontal pockets, our findings further suggest that H_2S may be an important trigger of tissue damage under conditions of periodontal disease.

Acknowledgments

This study was supported by NIH grant DE-13819. We thank Dr Howard Kuramitsu for reviewing this manuscript and Dr John Rugh and Dr Xiaoping Xu for their excellent discussion.

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