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The sensitivity of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* to different (pseudo)halide-peroxidase combinations compared with mutans streptococci

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Previous studies have shown that the peroxidase system with iodide is particularly effective against *Actinobacillus actinomycetemcomitans*. In the present study, the effects of iodide, chloride and thiocyanate in combinations with lactoperoxidase (LP) and myeloperoxidase (MP) on the viability of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Streptococcus mutans* and *S. rattus* were analysed. Bacteria were incubated in buffer solution containing peroxidase, substrate(s) and H$_2$O$_2$ (all in oral physiological concentrations), and plated after 0, 0.5 and 1 h. The oxidation product of iodide was the most bactericidal against all the bacteria tested. The effect was significantly weaker on mutans streptococci. Physiological concentrations of thiocyanate abolished the effects of LP-H$_2$O$_2$-iodide and MP-H$_2$O$_2$-iodide/chloride combinations. Thiocyanate-peroxidase systems have already been used in oral hygiene products. The incorporation of iodide into these products could make them much more potent against periodontal pathogens, and also help to prevent transmission of these pathogens from person to person via saliva.

Introduction

Human oral fluids, saliva and gingival crevicular fluid, contain peroxidase enzymes which are part of the innate host defence system. Salivary peroxidase (SP) is secreted by major salivary glands and is found only in saliva, whereas myeloperoxidase (MP) originates from polymorphonuclear leukocytes (PMNLs) and filters into saliva from gingival crevicular fluid. The amount of MP in gingival crevicular fluid, and thereafter also in saliva, depends on the periodontal status [1]. In periodontally healthy persons, the amount of MP in whole saliva is c. 3.6 μg/ml, which is twice the amount of SP (1.9 μg/ml) [2]. The complete peroxidase system consists of three components: a peroxidase enzyme, hydrogen peroxide (H$_2$O$_2$) and an oxidisable substrate such as a halide or a pseudohalide. Both SP and MP can oxidise thiocyanate (SCN$^-$) and iodide (I$^-$), whereas chloride (Cl$^-$) is oxidised only by MP. In gingival crevicular fluid, Cl$^-$ is most abundant (c. 90 mM) and the amount of I$^-$ (4.3, SD 1.9 μM) is only about one-tenth of the SCN$^-$ concentration (37 SD 24 μM) [3, 4]. In stimulated whole saliva, the Cl$^-$ concentration is in the range 10–56 mM and the I$^-$ concentration is c. 10 SD 7 μM [5]. The salivary concentrations of SCN$^-$ vary and depend, for example, on diet and smoking habits. The normal range of salivary SCN$^-$ for non-smokers is 0.5–2 mM, but concentrations as high as 6 mM can be found in saliva from smokers [6, 7]. The peroxidase systems have been shown to inhibit bacterial [8–14], fungal [15, 16] and viral [17–19] viability.

*Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* are among the main pathogens in periodontitis. *Fusobacterium nucleatum* has been proposed as the major cause of initial periodontal irritation and it usually maintains its proportion in the periodontal flora when gingivitis progresses to periodontitis [20]. The complete lactoperoxidase (LP) system has been used in some oral hygiene products, mainly to prevent caries. Although the use of such products elevates the amount of hypothiocyanous acid/ hypothiocyanite ion (HO SCN$^-$/OSC N$^-$) in saliva [21],

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their effects on the levels of *Streptococcus mutans*, lactobacilli or the total oral flora *in vivo* have not been significant [21–23]. However, there is some evidence of their favourable influences on the condition of the gingiva in patients who suffer from oral dryness [22].

The effect of peroxidase systems on *A. actinomycetemcomitans* has been studied extensively [10, 13, 24, 25]. An earlier study demonstrated a superior effect of the oxidation product of I– compared with the other (pseudo)halides on *A. actinomycetemcomitans* [10]. However, *A. actinomycetemcomitans* is only one of the pathogens in periodontitis. Therefore, this study focused on *P. gingivalis* and *F. nucleatum*. These results were compared with previous and current findings on the susceptibility of *A. actinomycetemcomitans*, *S. mutans* and *S. rattus* to the peroxidase system. These results will help further development of antimicrobial oral hygiene products.

**Materials and methods**

**Bacteria and growth conditions**

A clinical isolate of *P. gingivalis* TUPg007 (kindly provided by Dr K. Kari, University of Helsinki, Finland) and *F. nucleatum* ATCC 10953 were grown on Brucella Blood Agar (Becton Dickinson, Cockeysville, MD, USA) with frozen sheep blood 5% and non-selective CVE-plates containing trypticase (Becton Dickinson) 1%, yeast extract (Difco, Detroit, Michigan, USA) 0.5%, NaCl 0.5%, glucose 0.2%, L-tryptophan 0.02%, Bacto-agar (Difco) 1.5%, frozen sheep blood 5%, respectively. Before each experiment, *S. mutans* ATCC 25175 and *S. rattus* ATCC 19645 were grown on Blood Agar (Gibco BRL, Paisley) with frozen sheep blood 5%. After incubation, *S. mutans* and *S. rattus* were plated on MS-plates containing Mitis-salivarius agar (Difco) 9% and potassium tellurite 15 mg/L. An anaerobic atmosphere (CO2 10%, N2 80%, H2 10%) and a temperature of 37°C were used for growth of the anaerobic bacteria (*P. gingivalis* and *F. nucleatum*) and a CO2-rich atmosphere (CO2 7%, O2 19%, N2 74%) at 37°C for *S. mutans* and *S. rattus*. Before each experiment, *F. nucleatum*, *S. mutans* and *S. rattus* were grown for 4 days and *P. gingivalis* for 6 days. The bacteria were suspended in the test media to OD600: 0.90 SD 0.01 for *P. gingivalis*, *S. mutans* and *S. rattus*, and 0.97 SD 0.02 for *F. nucleatum*, corresponding to c. 6.3 × 106, 1.7 × 107, 1.7 × 108 and 1.1 × 109 cfu/ml, respectively.

**Media and chemical assays**

Solution I [10] was used in the experiments with I– and SCN–. It contained 9 mM Na2HPO4, 24 mM KH2PO4, 1.5 mM MgSO4 and 67 mM Na2SO4. When Cl– was used as a substrate, Solution II (9 mM Na2HPO4, 24 mM KH2PO4, 1.5 mM MgSO4) was used. The pH of the media was adjusted to 6.5.

LP and MP activities were analysed by Nbs-SCN assay [26]. In the assay, peroxidases in the sample oxidise 4.0 mM SCN– in the presence of 100 μM H2O2 to produce hypoiodous acid (HIO). OCN– ions oxidise 5-thio-2-nitrobenzoic acid (TNB), which is produced from 5,5′-dinitrobis-(2-nitrobenzoic acid) (DTNB) by reduction with 2-mercaptoethanol. The TNB concentration was adjusted to c. 50 μM with 2-mercaptoethanol. The absorbance of the reaction mixture was determined at 412 nm before and 15 s after the addition of H2O2, and the amount of oxidation product produced was counted from the decrease in absorbance: 1 μM corresponds to 1 μM oxidising product of TNB produced in 1 min. Stock solutions of LP and MP were prepared and the activity was determined. The peroxidase stock solutions were divided into tubes and kept frozen (−20°C) until used. Both LP and MP sustained their activities when stored at −20°C in phosphate buffer.

**Determination of the effective H2O2 concentration**

*P. gingivalis* was chosen as a test bacterium because of its greater sensitivity to the peroxidase systems than *F. nucleatum*, as observed in preliminary studies. The H2O2 concentrations were varied 0–40 μM and 0–10 μM in tests with LP-SCN– (1 mM) and LP-I– (5 μM) combinations, respectively. The LP concentration was 5 μg/ml. The bacteria were incubated for 30 min with LP and either SCN– or I– and the appropriate concentration of H2O2, then plated on Brucella blood agar and numbers of cfu were determined as described previously [10].

**Effects of the peroxidase systems on the viability of bacteria**

The antibacterial effects of different (pseudo)halide combinations of I–, Cl– and SCN– as substrates of both LP and MP systems were examined as described in detail by Ilahin et al. [10]. Bovine LP, RZ = 0.74 (Sigma) and human MP, RZ = 0.76 (a generous gift from the late Professor B. Mässon-Rahentullla, Birmingham, AL, USA) were used at a final concentration of 5 μg (22 mU)/ml and 4 μg (22 mU)/ml, respectively. The I– concentration was 5 μM, the SCN– concentration was either 50 μM or 1 mM and the H2O2 concentration was 1.25 μM or 5 μM. After incubation of bacteria, and the appropriate components of the peroxidase system, on a shaking water bath at 37°C for 1 h, 5 μl of a reducing reagent, diiihotretiol (DTT), were added to a final concentration of 1 mM to abolish the oxidation. Samples were taken immediately before the addition of H2O2 (0 min) and after incubation for 30 and 60 min. Ten-fold dilutions of the samples were made in peptone water containing DTT – tryptone (Difco) 2.5%, thionite E peptone (Becton Dickinson) 2.5%, NaCl 5%, DTT 0.02% – in all experiments with anaerobic bacteria. In experiments with *S. mutans* and *S. rattus*, DTT was excluded from the dilution medium.
After each experiment, dilutions were plated to enumerate the number of cfu and the growth conditions were as described previously. The incubation time for P. gingivalis was 7 days, for F. nucleatum 4 days and for S. mutans and S. ratus 3 days. Each experiment was repeated three times and the means of log$_{10}$ cfu, as well as standard deviations (SD), were counted. A control without H$_2$O$_2$ was included in every experiment. The effects of the peroxidase systems on the bacteria in the presence of DTT were examined as described previously [10] and every substrate (I$^-$, Cl$^-$, SCN$^-$) was used separately.

Statistical analysis

The reduction of the number of bacteria (log$_{10}$ cfu/ml) was analysed by Student's paired two-tailed t test. Differences in log$_{10}$ cfu between the test systems and the controls after incubation for 0, 30 and 60 min were analysed and p values <0.05 were considered to be statistically significant.

Results

Effects of different H$_2$O$_2$ concentrations in LP systems

The effects of both LP-SCN$^-$ and LP-I$^-$ systems on the viability of P. gingivalis were dose-dependent with respect to H$_2$O$_2$. The LP-I$^-$ system with 2.5 $\mu$m H$_2$O$_2$ killed all the bacteria within 30 min, whereas only a slight decrease in log$_{10}$ cfu/ml (from 7.8 to 6.6) was detected with the same amount of H$_2$O$_2$ in the LP-SCN$^-$ system. Even a H$_2$O$_2$ concentration as high as 40 $\mu$m with LP and SCN$^-$ did not totally inhibit the growth of P. gingivalis; after incubation for 30 min the log$_{10}$ cfu/ml was still 5.4 SD 0.1.

Effects of the myeloperoxidase systems

The effects of MP systems with different (pseudo) halide combinations were tested with the gram-negative anaerobic bacteria, P. gingivalis and F. nucleatum. Both bacteria showed similar susceptibility and the oxidation product of I$^-$ was found to be the most effective antimicrobial agent, followed by Cl$^-$ and SCN$^-$ (Table 1). The addition of SCN$^-$ diminished the effect of the MP-I$^-$ system to the same level that was achieved with SCN$^-$ as the only substrate. When all these three substrates were combined, the bactericidal effect of the MP system was similar to the effect of the MP-SCN$^-$ system. The concentrations of substrates were: 5 $\mu$m I$^-$; 90 $\mu$m Cl$^-$; 50 $\mu$m SCN$^-$ and 1.25 $\mu$m H$_2$O$_2$. Moreover, the addition of SCN$^-$ abolished the bactericidal effect of the MP-H$_2$O$_2$ (5 $\mu$m)-Cl$^-$ (90 mM) system (data not shown). The control reaction with 5 $\mu$m I$^-$, 90 mM Cl$^-$ and MP 5 $\mu$g/ml without H$_2$O$_2$ reduced the viability of F. nucleatum more than the other controls with different (pseudo)halide combinations; therefore, this control is shown separately in Table 1.

Effects of the lactoperoxidase systems

The effects of the LP systems were tested on both gram-negative anaerobes (P. gingivalis and F. nucleatum) and gram-positive aerobes (S. mutans and S. ratus). The concentrations of substrates were 5 $\mu$m I$^-$, 1 mM SCN$^-$ and 1.25 $\mu$m H$_2$O$_2$. The oxidation product of I$^-$ was most effective against all bacteria tested.

Table 1. Effects of myeloperoxidase (4 $\mu$g (22 U)/ml) systems with different substrates on the viability of P. gingivalis and F. nucleatum after incubation for 0 and 60 min at 37°C, pH 6.5

<table>
<thead>
<tr>
<th>Halide</th>
<th>P. gingivalis</th>
<th>F. nucleatum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>60 min</td>
</tr>
<tr>
<td>I$^-$</td>
<td>7.2 (0.5)</td>
<td>6.5 (0.4)</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>7.6 (0.1)</td>
<td>6.9 (0.1)</td>
</tr>
<tr>
<td>SCN$^-$</td>
<td>7.2 (0.3)</td>
<td>6.8 (0.2)</td>
</tr>
<tr>
<td>I$^-$/SCN$^-$</td>
<td>7.0 (0.2)</td>
<td>6.7 (0.3)</td>
</tr>
<tr>
<td>I$^-$/Cl$^-$</td>
<td>7.6 (0.2)</td>
<td>6.8 (0.1)</td>
</tr>
<tr>
<td>I$^-$/Cl$^-$</td>
<td>7.5 (0.1)</td>
<td>6.9 (0.0)</td>
</tr>
<tr>
<td>Control</td>
<td>7.4 (0.3)</td>
<td>6.8 (0.3)</td>
</tr>
<tr>
<td>Control I$^-$/Cl$^-$</td>
<td>- - -</td>
<td>6.9 (0.1)</td>
</tr>
</tbody>
</table>

The substrate concentrations were: 5 $\mu$m I$^-$, 90 mM Cl$^-$; 50 $\mu$m SCN$^-$ and 1.25 $\mu$m H$_2$O$_2$.

*Values are shown as means (SD) of three-to-nine different experiments with p values of Student's paired two-tailed t test.

†Mean (SD) of all combination control reactions (i.e., with no H$_2$O$_2$) except the control I$^-$/Cl$^-$ (F. nucleatum).

‡Control reactions including: 5 $\mu$m I$^-$, 90 mM Cl$^-$ and MP 5 $\mu$g/ml with no H$_2$O$_2$.
The anaerobic gram-negative bacteria (P. gingivalis and F. nucleatum) showed similar susceptibility to all the (pseudo)halide-LP systems tested, as in experiments with MP (data not shown). The gram-positive bacteria (S. mutans and S. rattus) were more resistant to LP systems and, with S. rattus, there was no statistically significant inhibitory effect of I− oxidation. The effect of the oxidation product of I− on S. mutans seemed to be transient (Table 2). However, when the concentration of H2O2 was increased from 1.25 μM to 5 μM, S. mutans could not recover from the effect of the LP-H2O2-I− system (Table 3).

The experiments with P. gingivalis and F. nucleatum were also done with 50 μM SCN−, but the results did not differ from those in experiments with 1 mM SCN− (data not shown).

Effects of peroxidase systems with DTT on the viability of bacteria

With all (pseudo)halides and P. gingivalis and F. nucleatum, the bactericidal effect of the peroxidase systems was abolished after adding DTT. The decrease of log10 cfu/ml was inhibited both when DTT was added at the beginning (0 min) or after incubation for 30 min. No recovery of the number of cfu was observed after incubation for 30 min with DTT (Fig. 1).

Discussion

Gram-negative obligatory or facultatively anaerobic oral bacteria are located mainly in gingival crevices. However, they are able to transmit within a family from parent to child and between spouses [27–29], and saliva is thought to be the route in these transmissions. Peroxidase (SP and MP) systems are part of the innate host defence in human saliva [30] and they affect the viability of various bacteria including the gram-negative bacteria A. actinomycesecomitans [10, 13, 24, 25], P. gingivalis [31] and F. nucleatum [8] in vitro. A recent study showed that in LP, which is structurally and catalytically close to SP [32], and MP systems, the oxidation product of I− was most effective against A. actinomycesecomitans, and that the addition of physiological concentrations of SCN− abolished this inhibitory effect [10]. As there are several pathogens in

Table 2. Effects of lactoperoxidase (5 μg (22 μM)/ml) systems with different substrates on the viability of S. mutans and S. rattus after incubation for 0, 30 and 60 min at 37°C, pH 6.5

<table>
<thead>
<tr>
<th>Halide</th>
<th>S. mutans</th>
<th>S. rattus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>I−</td>
<td>7.8 (0.0)</td>
<td>5.1 (0.5)</td>
</tr>
<tr>
<td>p = 0.018</td>
<td>p = 0.082</td>
<td>p = 0.065</td>
</tr>
<tr>
<td>SCN−</td>
<td>7.8 (0.1)</td>
<td>7.9 (0.2)</td>
</tr>
<tr>
<td>p = 0.29</td>
<td>p = 0.57</td>
<td>p = 0.58</td>
</tr>
<tr>
<td>I−+SCN−</td>
<td>7.7 (0.1)</td>
<td>7.6 (0.1)</td>
</tr>
<tr>
<td>p = 0.66</td>
<td>p = 0.95</td>
<td>p = 0.54</td>
</tr>
<tr>
<td>Control 2</td>
<td>7.9 (0.1)</td>
<td>7.6 (0.2)</td>
</tr>
</tbody>
</table>

The substrate concentrations were: 5 μM I−; 1 mM SCN− and 1.25 μM H2O2.

Table 3. Effects of lactoperoxidase (5 μg (22 μM)/ml) systems with different substrates on the viability of S. mutans and S. rattus after incubation for 0, 30 and 60 min at 37°C, pH 6.5

<table>
<thead>
<tr>
<th>Halide</th>
<th>S. mutans</th>
<th>S. rattus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>I−</td>
<td>7.8 (0.3)</td>
<td>3.3 (0.8)</td>
</tr>
<tr>
<td>p = 0.0024</td>
<td>p = 0.040</td>
<td>p = 0.0024</td>
</tr>
<tr>
<td>SCN−</td>
<td>7.8 (0.2)</td>
<td>7.6 (0.5)</td>
</tr>
<tr>
<td>p = 0.77</td>
<td>p = 0.32</td>
<td>p = 0.77</td>
</tr>
<tr>
<td>I−+SCN−</td>
<td>7.8 (0.3)</td>
<td>7.6 (0.6)</td>
</tr>
<tr>
<td>p = 0.96</td>
<td>p = 0.37</td>
<td>p = 0.29</td>
</tr>
<tr>
<td>Control 2</td>
<td>7.8 (0.3)</td>
<td>7.6 (0.4)</td>
</tr>
</tbody>
</table>

The substrate concentrations were: 5 μM I−; 1 mM SCN− and 5 μM H2O2.

*Values are shown as means (SD) of three different experiments with p values of Student's paired two-tailed t test.

1Mean (SD) of all combination control reactions (i.e., with no H2O2).
periodontitis, the present study investigated the effects of LP and MP systems with different (pseudo)halide combination on *P. gingivalis* and *F. nucleatum*. These effects were compared with gram-positive control bacteria, *S. mutans* and *S. rattus*. Physiological concentrations of (pseudo)halides and peroxidases were used. Previous studies on *A. actinomycetemcomitans* and *S. mutans* have shown that these halide concentrations, in combination with LP/MP and H₂O₂, cause significant antibacterial effect [10,14], and that the peroxidase activities rapidly produce OSCN⁻ from SCN⁻ in the presence of H₂O₂ [33]. H₂O₂ concentration is not measurable in saliva, because it is actively produced and depleted in biochemical reactions. However, its concentration in whole saliva has been estimated to be in the range 8 – 13 μM [34]. The H₂O₂ concentrations used in this study were somewhat lower than the estimated physiological range, but they were adjusted to cause statistically significant inhibition of bacterial viability with the most efficient antimicrobial peroxidase-halide combination.

In this study, the oxidation product of I⁻ (with LP and MP) was again found to be the most effective antimicrobial agent, followed by the oxidation products of Cl⁻ (with MP) and SCN⁻ (with LP and MP) against all bacteria tested. *P. gingivalis* and *F. nucleatum* showed similar susceptibility to all peroxidase systems tested. The results support the findings that *P. gingivalis* is susceptible to the LP-SCN⁻ system in the presence of H₂O₂ [31]. However, the amount of H₂O₂ used with LP/MP did not produce enough OSCN⁻ to kill *F. nucleatum* effectively in comparison with a previous study in which OSCN⁻ concentrations above 8 μM killed >75% of bacteria [8]. Both gram-negative bacterial species were more sensitive to the MP-H₂O₂-Cl⁻ system than *A. actinomycetemcomitans* [10].

The effects of oxidised I⁻ and SCN⁻ were significantly weaker on *S. mutans* and *S. rattus* than on the gram-negative bacteria. The oxidation product of I⁻ (with LP), unlike that of SCN⁻, decreased the log₁₀ cfu of *S. mutans/ml* significantly. At low H₂O₂ concentration, the effect seemed to be only transient but *S. mutans* numbers did not recover when the concentration of H₂O₂ was increased. It has been shown that the peroxidase-H₂O₂ (126 μM)I⁻ (14 μM) system kills *S. mutans* at pH 7.2 [35]. However, the H₂O₂ concentration was 25 times higher than the concentration used in the present study. It has been shown with various bacteria that the effects of the complete peroxidase systems are dose-dependent with regard to H₂O₂ [10, 14]. *S. rattus* was even more resistant to the LP-H₂O₂-I⁻ system than *S. mutans*, and no statistically significant inhibition was observed.

The H₂O₂ concentration used in this study was obviously too low to affect the viability of *S. mutans* and *S. rattus* in the LP-H₂O₂-SCN⁻ system at pH 6.5. These results agree with the findings that LP-SCN⁻ (1 mM) system, even with concentrations as high as 80 μM H₂O₂, is not bactericidal against *S. mutans* at pH 7 after incubation for 1 h [36]. Thus, it is concluded that oral streptococci, *S. mutans* and *S. rattus*, are much more resistant to the antibacterial activity of saliva peroxidase systems than gram-negative anaerobes.

It was shown recently that SCN⁻ is the best electron donor for the MP compound I⁻ (oxidised by H₂O₂), followed by I⁻ and Cl⁻ [37]. When physiological amounts of SCN⁻ and Cl⁻ are combined, even with a 10²-fold excess of Cl⁻, MP converts 50% of H₂O₂ into HOCl instead of HOCl [38]. This may explain why physiological amounts of SCN⁻ abolished the anti-

**Fig. I.** Effects of LP-I⁻ (5 μM)-H₂O₂ (1.25 μM) system with 1 mM DTT on the viability of (a) *P. gingivalis* and (b) *F. nucleatum* at 37°C. DTT was added at 0 or 30 min. In control experiments, DTT was not added (No DTT). Samples were withdrawn at 0 min (■), 30 min (□) and 60 min (▲). Data are shown as values for one experiment. The detection limit was log₁₀ 2 cfu/ml. MP/Cl⁻ and LP/SCN⁻ systems gave similar results (data not shown).
bacterial effect of the MP/LP-H₂O₂·⁻ and MP-H₂O₂·⁻ systems with all bacteria tested, whereas Cl⁻ had no effect on the MP-H₂O₂·⁻ system. Similar results have also been reported with the MP-H₂O₂·⁻ system against S. mutans [14]. Thus, SCN⁻ most probably is the preferred substrate of SP.

There is intra-species variability in the susceptibility of various bacteria to the LP-H₂O₂·SCN⁻ system [8]. In this study, only one strain of each bacterium was investigated; hence, the susceptibilities of different strains of P. gingivalis and F. nucleatum require further study. Surprisingly, the F. nucleatum control with MP, H₂O₂, and Cl⁻, without H₂O₂, decreased the viability of the bacterium in a statistically significant manner. As the addition of DTT totally abolished this effect and the addition of H₂O₂-degrading catalase only partly, the presence of some oxidising substance other than H₂O₂ can be hypothesised. Moreover, similar controls with only H₂O₂ or Cl⁻ had no effect on viability.

MPO-produced HOCl is able to chlorinate ammonia and amines to yield chloramines, which act as carriers of the oxidised Cl⁻. Chloramines differ physically and chemically from HOCl and, therefore, their biological effects may be significantly different from those of HOCl [39]. Saliva and gingival crevicular fluid contain a wide variety of proteins and glycoproteins, which can act like amines, modifying the effects of peroxidase- H₂O₂-halide combinations in vivo. Preliminary studies indicate that the antibacterial effects of the peroxidase- H₂O₂-halide combinations investigated decrease in saliva.

It is concluded that peroxidase-catalysed oxidation products of H₂O₂ are effective against the tested anaerobic bacteria in vitro, as shown for various other bacteria and fungi [9, 10, 40, 41]. In fact, the effect is considerably stronger than that of the LP-H₂O₂-SCN⁻ system, which is used in some oral hygiene products [21, 42]. Therefore, the addition of I⁻ to saliva might improve the bactericidal properties of this system and also prevent the transmission of periodontal bacteria via saliva. However, as was shown also in this study, the presence of physiological concentrations of SCN⁻ significantly diminishes the antibacterial properties of the LP/MP-H₂O₂-I⁻ system [10, 40]. To circumvent the problem of competitive substrates, the amount of I⁻ may be increased further. Furthermore, the presence of different proteins in saliva may also affect the bactericidal activity of the peroxidase systems in vivo. The present study investigated the bacterial sensitivities in buffer solution and, thus, the effects of the peroxidase systems in saliva need further studies.

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