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REVIEW ARTICLE

The role of hypochlorous acid as one of the reactive oxygen species in periodontal disease

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Hypochlorous acid (HOCl) has both proinflammatory and anti-inflammatory properties, and seems to play an important role in the immune system. The regulation of normal flora contributes to periodontal health, and HOCl seems to have the ability to attack Gram-negative pathogens during periodontitis. Furthermore, high concentrations of HOCl promote healing by regulating cytokines and growth factors, killing pathogens through chlorination or oxidation, and modulating inflammation through the effects on nuclear factor κB and activator protein-1 of monocytes. After chlorination of taurine by HOCl, taurine chloramine is mostly an anti-inflammatory agent and enhances healing. Neither HOCl nor taurine chloramine are common in clinical applications owing to a lack of studies in animal and human models. Both compounds may be suitable as periodontal medication, as they are good antimicrobial agents, inflammation modulators, and healing promoters.

Introduction

Periodontitis is an inflammatory process initiated by plaque biofilm that leads to loss of periodontal attachment to the root surface and adjacent alveolar bone, and which ultimately results in tooth loss.[1] Destruction of periodontal tissue in periodontal disease is caused by an inappropriate and exaggerated host response to certain microorganisms and their products.[2,3] Thus, regulation of the host response, including immune cells and cytokines, is crucial in treating periodontitis.

Free radicals are any species capable of independent existence that contain one or more unpaired electrons[3] and are able to oxidize a variety of biomolecules and cell components that are vital to cell and tissue function. Reactive oxygen species (ROS) include not only free radicals but also other reactive species, which are not true radicals but are capable of radical formation in either an intra- or extracellular environment.[1] Hypochlorous acid (HOCl), as one of the ROS, is released by neutrophils in periodontal pockets, and seems to have a role in regulating inflammation and healing in destroyed gingival and periodontal tissues.[2] Yet, therapeutic use of HOCl in periodontitis has not been well studied. Further studies on HOCl as an antimicrobial and health-promoting medication may contribute to a better

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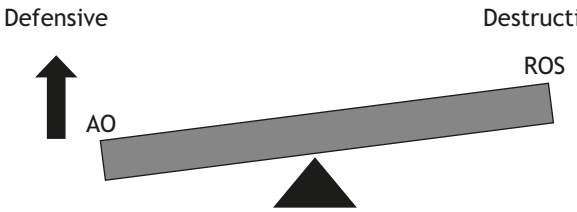


Fig. 1 In normal physiologic conditions, the antioxidant (AO) defense system is always superior to the reactive oxygen species (ROS) system.

prognosis for periodontitis treatment. Taurine chloramine (TauCl), a scavenging product of HOCl, has some of the properties of HOCl, and it seems to promote healing.[2]

Oxidative stress and the redox state

In normal physiology, the dynamic equilibrium between ROS activity and the antioxidant defense system, which protects and repairs vital tissues, cells and molecular components, shifts to a defensive trend (Fig. 1).

Oxidant stress was defined by Sies[4] as "a disturbance in the pro-oxidant-antioxidant balance in favor of the former, leading to potential damage". It occurs when there are more ROS than defensive antioxidation, owing to either a reduction in antioxidants or an increase in ROS production.[1] Direct tissue damage results.

A low redox potential or a reducing environment within periodontal cells and tissues is protective against oxidative stress and helps maintain good health. However, when a periodontal pathogen is present, components or toxins of the pathogen stimulate the production of ROS by polymorphonuclear neutrophils (PMNs),[5] thus promoting oxidative stress in the periodontal sulcus or pocket.

Origins of ROS in a normal physiologic state

One of the origins of ROS is mitochondria. During cell metabolism, oxygen is consumed in glycolysis to generate energy, forming pyruvate within the mitochondria. Electrons produced from mitochondrial electron transport systems (respiratory chains) form superoxides at a constant rate as a byproduct of the metabolic pathway. The mitochondrial enzyme, manganese-dependent superoxide dismutase (MnSOD), scavenges superoxide radicals, converting them into hydrogen peroxide (H_2O_2). However, mitochondrial antioxidant scavengers are sometimes unable to safely take care of all of the ROS produced. Mitochondria are more susceptible than nuclear DNA to damage by ROS[6] because of their proximity to the ROS generated, the lack of histone proteins to scavenge radicals, and perhaps inefficiencies in the poly(adenosine diphosphate-ribose) polymerase DNA repair mechanism which repairs strand breaks.[7]

The superoxide radical anion ($O_2^{\bullet -}$), the primary product of oxygen metabolism in the mitochondrial respiratory chain, is formed by the hexose-monophosphate shunt, via the transfer of one electron to molecular oxygen. Another possible reason for the weak scavenging ability of mitochondria is that they lack catalase. Neutralization of H_2O_2 into H_2O is carried out by the enzyme glutathione peroxidase, with the requirement of a coenzyme-reduced glutathione.

Formation of ROS by PMNs

PMNs are the primary mediators of a host's response to pathogenic microbes during inflammatory diseases. Some of the antimicrobial factors produced by PMNs are capable of preventing bacterial growth through their antibacterial properties.[8] During inflammation especially chronic inflammation, inflammatory cells such as activated macrophages and PMNs release various ROS (H_2O_2, nitric oxide [NO], $O_2^{\bullet -}$, and $OH^{\bullet}$) and HOCl.[9] Some of the antimicrobial agents produced by PMNs are ROS, which provide a protective role for the host against inflammation.

Different kinds of mitogens, antigens (e.g., small peptides from bacteria), cytokines and mediators, like granulocyte-macrophage colony-stimulating factor, promote the formation of ROS by PMNs. The activation of the Fcγ receptor by opsonized particles, or toll-like receptors (e.g., TLR-4 and TLR-9) by bacterial DNA triggers ROS formation.[10]

In a zone of the PMN plasma membrane contacting the phagocytosed particle, $O_2^{\bullet -}$ is produced by activation of the hexose monophosphate shunt, in which molecular oxygen is catalyzed by NADPH oxidase. This process comprises the respiratory burst within PMNs. The elevated Ca^{2+} in PMNs, resulting from cell surface receptor stimulation, opens Ca^{2+}-dependent K^+channels in the phagolysosome (vacuole) membrane. Superoxide is pumped into the vacuole by NADPH oxidase, depolarizing the membrane and generating an ionic gradient. The occurrence of K^+influx, creating a hypertonic K^+-rich alkaline environment within the vacuole, activates lysosomal proteases, leading to microbial destruction.[11]

Superoxide radical anions are reduced to H_2O_2 by superoxide dismutase (SOD; 1 of 3 forms). SOD is localized within human periodontal ligaments and may play a role in defense against excessive superoxide release.[12]

Hydrogen peroxide, NO, superoxide radicals, and hydroxyl radicals are produced by mitochondria,

leading to elevated numbers and activity of PMNs. In addition to those already mentioned, HOCl can be produced by PMNs during inflammatory conditions.

Effects of ROS on periodontal tissues and cell components

Stimulated by bacterial antigens in periodontal tissue, ROS produced by PMNs increase oxidative damage in gingival tissues, periodontal ligaments, and alveolar bone.[13] ROS damage periodontal tissues and cell components by depolymerization of extracellular matrix (ECM) components,[14,15] lipid peroxidation,[16] oxidation of defensive or protective enzymes (e.g., anti-protease),[16] increased apoptosis in the deepest area of the sulcular pocket,[17] activation of osteoclasts,[18,19] induction of proinflammatory cytokines, and DNA damage.[16]

ECM components

Excessive ROS produced by PMNs destroy ECM components and alter the metabolic reaction responsible for synthesis of ECM components. Proteoglycans (PGs) and glycosaminoglycans (GAGs), which are some of the ECM components, have been shown to regulate mineralization[20,21] and cellular function by their ability to bind growth factors, such as transforming growth factor β.[22] Thus, the overproduction of ROS degrades PGs by modifying amino acid functional groups, leading to fragmentation of the core protein, while the constituent GAG undergoes limited depolymerization. Sulfated GAGs are more resistant than non-sulfated GAGs, such as hyaluronan, to ROS degradation *in vitro*.[23,24]

Another important ECM component is type I collagen. Containing 1000 amino acids that form a triple helix, it resists nonspecific proteolytic attacks. ROS directly attack collagen, which results in fragmentation, and makes it more susceptible to breakdown by collagenase.[24,25] Superoxide anions and hydroxyl radicals are able to cleave collagen into small peptide fragments at the proline and hydroxyproline residues.[26] Furthermore, lipid peroxidation products (e.g., malondialdehyde) of ROS may interact with collagen and alter fibroblast functions.[27] This phenomenon can be seen within gingival tissues of patients with periodontal disease as lipid peroxidation increases.

Activation of osteoclasts

Although the effects of ROS on bone resorption are not well documented or studied, it has been shown that certain ROS (i.e., superoxide and H_2O_2) promote osteoclast formation.[28] More substantial evidence supporting this theory is that osteoclasts produce ROS at the ruffle border/bone interface.[29,30] Chondroitin sulfate PGs from alveolar bone are particularly susceptible to damage by hydroxyl radicals *in vitro*.[15] In short, ROS may worsen bone destruction in periodontal disease.

DNA damage

Mitochondrial DNA is the most susceptible molecule attacked by ROS. Deletions of mitochondrial DNA by ROS are associated with aging and several chronic diseases,[31] including chronic periodontitis. Five kilobase of mitochondrial DNA deletions was found in gingival tissues of patients with chronic adult periodontitis.[32] Once the mitochondrial DNA is damaged, oxidative stress within a cell can be amplified owing to an abatement of the expression of proteins which are crucial for electron transport, resulting in cell death.

Transformation and chlorination of H_2O_2 to HOCl (Fig. 2)

When a pathogen invades periodontal tissues, PMNs are activated. Phagocytosis of the pathogen by PMNs results in assembly and activation of the respiratory burst NADPH oxidase in the phagolysosome membrane. The oxidase reduces O_2 to $O_2^{\bullet-}$ and releases $O_2^{\bullet-}$ into phagolysosomes. The spontaneous dismutation or SOD catalysis of two $O_2^{\bullet-}$ radicals produces O_2 and H_2O_2. Myeloperoxidase catalyzes the oxidation of chloride ions by H_2O_2 to produce HOCl, and the reaction of HOCl with amines and ammonium ions produces toxic chloramines such as monochloramine (NH_2Cl). The O_2 metabolites (i.e., $O_2^{\bullet-}$ and H_2O_2) and products of chloride oxidation (i.e., HOCl and NH_2Cl) contribute to the antimicrobial activity of phagolysosomes.

HOCl, the end-product of PMN respiratory bursts, results from the intracellular myeloperoxidase-catalyzed reduction of H_2O_2 by chlorine during inflammation.[2] Myeloperoxidase is a lysosomal protein stored in the azurophilic granules of neutrophils. Once HOCl is formed by PMNs, it is released extracellularly.[33] In addition to intracellular myeloperoxidase, PMNs can secrete HOCl extracellularly.[33]

Immunologic effects of HOCl

Antibacterial properties of HOCl

HOCl-induced oxidation and/or chlorination may neutralize harmful bacterial endotoxins or exotoxins,

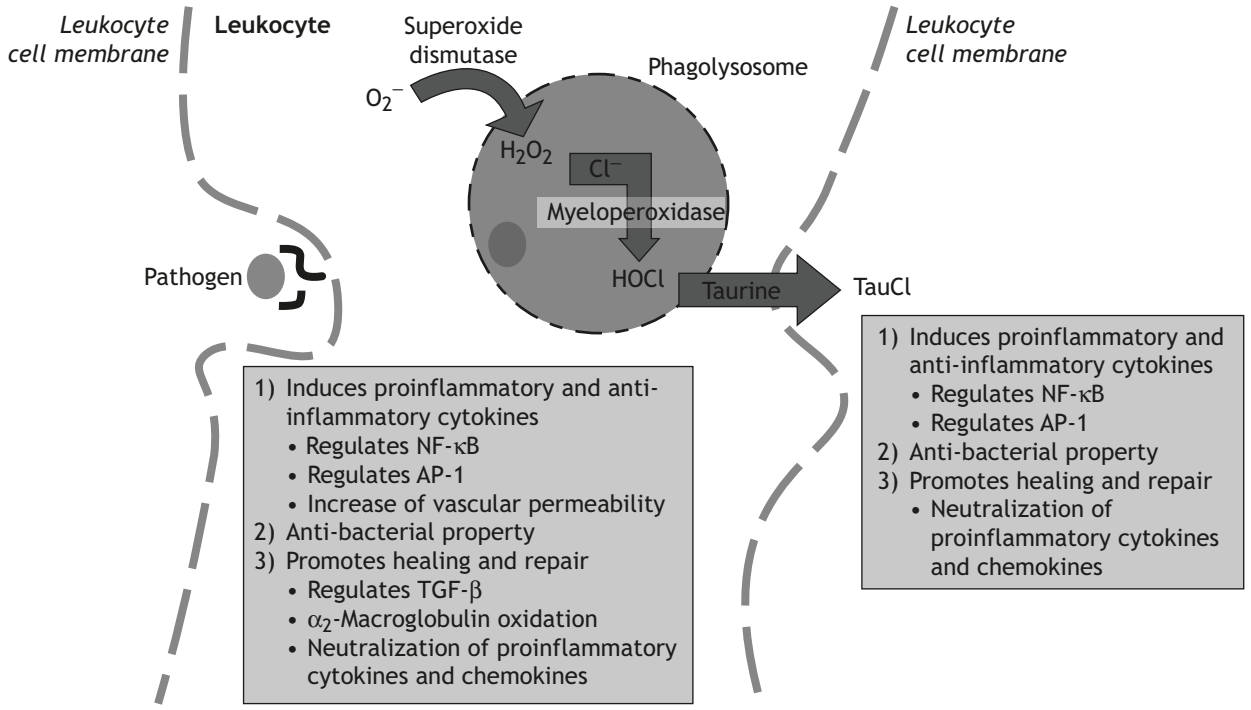


Fig. 2 Transformation and chlorination of hydrogen peroxide (H_2O_2) to hypochlorous acid (HOCl), and the reaction of HOCl with taurine. The gray boxes summarize the function of HOCl and taurine chloramine (TauCl).

such as lipopolysaccharide (LPS) and gingipains.[34] HOCl oxidizes the crucial cysteine residue of the active site of the gingipains, Rgp and Kgp. Both of them are cysteine proteases of *Porphyromonas gingivalis*, which lead to the destruction of periodontal tissues; thus, HOCl reduces their potentially harmful activity against periodontal tissues.[35]

Within physiologic concentration ranges, HOCl has immediate and highly effective microbicidal activity *in vivo*.[36] Various bacterial respiratory electron transporters are irreversibly oxidized by HOCl. HOCl may repulse some motile bacteria, especially those with flagella and gliding properties; however, the mechanism of this repulsive activity remains unclear.[37] HOCl is able to enhance the immunogenicity of antigens by HOCl chlorination of the proteinaceous parts of the antigens; this promotes the presentation of these proteins to antigen-presenting cells, such as monocytes, macrophages, and dendritic cells.[34]

Although the mechanism has not yet been clearly elucidated, chlorination of antigens selectively promotes a nonspecific immune response against Gram-negative periodontal pathogens, and reduces the response induced by Gram-positive bacteria. This affects the antigen phagocytosis-activated production of inflammation mediated by macrophages. The chlorination of Gram-positive bacteria-released antigens significantly affects macrophage secretory activities, such as decreasing NO and tumor necrosis factor (TNF)-α; however, phagocytosis and interleukin (IL)-6 production remain unchanged.[38] This may be critical in maintaining a normal physiologic state of periodontal tissues. The normal subgingival flora in periodontally healthy individuals mainly consists of Gram-positive bacteria, while potent periodontal pathogens are mostly Gram-negative bacteria, such as *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans*. As the defensive ability against Gram-negative bacteria is greater than that against Gram-positive bacteria, the immune system seems capable of protecting periodontal tissues by this mechanism. If the amount of TNF-α production is great with normal Gram-positive flora, the destruction may also occur in periodontally healthy individuals, showing the importance of TNF-α in destructive periodontitis.

The antimicrobial activity of HOCl has been extensively studied. It causes respiratory loss in bacterial cell membranes due to an irreversible reaction with sulfur- and heme-containing membrane enzymes and structural proteins,[39] leading to cell death and nonviability.[40]

Dose-dependent inflammatory response modulation

HOCl seems to play a key role in this regulation of proteinase activity, dysregulation of which may lead to periodontitis through a pathway distinct from that of tissue inhibitors of matrix metalloproteinases, and appears to reduce the activity of proteolytic enzymes in a concentration-dependent manner.[41,42]

Low concentrations of HOCl may be destructive to periodontal tissue. A low dose of HOCl can activate the proforms of matrix metalloproteinases (MMPs), collagenase-2, and gelatinase B via thiol group oxidation of their cysteine moiety, while higher concentrations of HOCl inhibit MMP-7 activation through an oxidative modification of adjacent tryptophan and glycine residues in the catalytic domain.[41] Similarly, HOCl inhibits collagenase activities, when the HOCl/collagenase ratio exceeds 40 (which indicates a high concentration of HOCl). HOCl may also inactivate gelatinases when the HOCl/gelatinase ratio exceeds 30, while it does not seem to inhibit them when the ratio is <30.[43,44]

Thus, HOCl possesses both pro- and anti-inflammatory properties depending on the dose. This may be a very important mechanism modulating the inflammatory response within periodontal tissues. HOCl is capable of mediating the generation of histamine *N*-chloramines, and thus may modulate histamine activity, tissue distribution, and metabolism within sites of inflammation.[45] Chemotactic mediators enhance leukocyte adherence to activated endothelium and *in situ* diapedesis.

Furthermore, HOCl neutralizes various proinflammatory cytokines and chemokines (chemotactic factors, leukotrienes, TNF-α, IL-1β, IL-2, and IL-6), regulates metalloproteinases, and releases activated growth factors. These reactions may be due to either a direct oxidation of crucial thiol or thioether residue(s) of the molecules or an indirect modulatory effect on the capacity of α_2-macroglobulins to bind them.

α_2-Macroglobulins are plasma molecules that bind to and neutralize proteases, cytokines, and growth factors. In plasma in a normal physiologic state, α_2-macroglobulin's binding affinity is greater toward growth factors (e.g., TGF-β, basic fibroblast growth factor [also called FGF-2], β-nerve growth factor [β-NGF], and platelet-derived growth factor [PDGF], with dissociation constant (K_d) values in a nanomolar range) than to cytokines (e.g., TNF-α, IL-1β, IL-2, IL-6, IL-8, with K_d values in a micromolar range), which leads to the activities of cytokines being more predominant than those of growth factors. Consequently, 85–90% of TGF-β and PDGF molecules are inactivated by being bound to α_2-macroglobulins. When α_2-macroglobulin is oxidized by HOCl, it tends to undergo repair and fibrosis, owing to a decrease in protease binding, an important increase in α_2-macroglobulin affinity for destructive or inflammation-induced factors (TNF-α, IL-2, and IL-6), and a greater decrease in affinity to growth factors (β-NGF, PDGF-BB, TGF-β1, and TGF-β2). In addition, after being oxidized by HOCl, the oxidized α_2-macroglobulin–methylamine complex leads to a decrease in the binding of various growth factors, resulting in increases in free growth factors, with no modification of its affinity to inflammatory cytokines.[46]

However, HOCl may exert a deleterious stimulation of inflammatory processes. As mentioned above, low concentrations of HOCl may activate the proforms of MMPs, gelatinase B, and collagenases. HOCl may also inhibit α_2-macroglobulin-related neutralization of cell proteases, whereas HOCl inactivates the α_1-proteinase inhibitor.[46,47] HOCl may also interfere with the C5 component of the complement cascade which, upon activation, generates two fragments: the C5b fragment with antibacterial membrane-lytic activity, and the C5a fragment with PMN chemotactic properties. HOCl-induced oxidation of methionine residues in the C5 fragment generates structural changes that result in its activation.[48] HOCl promotes macrophage adherence to the endothelium and enhances endothelial permeability.[49] HOCl promotes an innate immune response against Gram-negative bacteria (unlike Gram-positive species) via chlorination of antigens.[38]

In conclusion, unlike natural TNF-α or TNF-β, HOCl has a double-edged effect in periodontitis. Although the effects of HOCl in periodontitis are not well documented, HOCl seems to play a very important role in modulating the progress of periodontitis and may help in repairing its damage. With further studies and evidence, HOCl may become a drug used in surgery or postsurgical medication.

Enhanced cell proliferation and extracellular component production by HOCl

The interruption of tissue destruction, which promotes healing, is brought about by direct neutralization and cellular inhibition of proinflammatory mediators. HOCl also induces the production of cellular growth factors, such as insulin-like growth factor, epidermal growth factor, keratinocyte growth factor (also called FGF-7), FGF-1, FGF-2, TGF-β, PDGF, vascular endothelium growth factor, connective tissue growth factor, and/or cementum-derived growth factor.[50] These molecules also promote periodontal tissue regeneration. Signal transduction enzymes are involved in the synthesis of some of the above molecules.

Furthermore, HOCl can activate the transforming growth factor TGF-β, a reparative mediator which promotes tissue repair and fibrosis. Native TGF-β consists of two peptides: an *N*-terminal latency-associated peptide and a *C*-terminal mature TGF-β. HOCl-induced latency-associated peptide oxidation may facilitate access to the active site of the mature TGF-β molecule, resulting in its activation.

HOCl may also affect the signal transduction pathway in inflammatory cytokine formation. The nuclear factor κB and activator protein (AP)-1 are redox-sensitive transcription factors, the control of which has been proposed as a potentially important host-modulation strategy in periodontitis.[51] NF-κB and AP-1 are stimulated by a specific mitogen-activated protein (MAP) kinase pathway, which consists of a cascade of transduction signals activated by a membrane receptor-linked protein tyrosine kinase. In short, their activation and phosphorylation in periodontal tissues cause periodontitis.

NF-κB is an important signal transduction protein responsible for the genetic transcription of many inflammatory mediators, including those regulating inflammation, acquired immunity, cell-to-cell interactions, cell apoptosis, and proliferation (e.g., IL-1α, IL-1β, IL-2, IL-6, TNF-α, NO, prostaglandin E_2, TGF-β, and adhesion molecules), as well as inhibitors of apoptosis proteins. Inactive NF-κB forms a dimer with an IκB inhibitory protein in the cytoplasm, which masks its nuclear location signal site and latently stabilizes NF-κB. NF-κB is activated by specific MAP kinases, IκB kinases (IKKs). This inducible serine phosphorylation leads to a polyubiquitination of adjacent lysines in the IκB inhibitory protein, which is a signal of degradation in the 26S proteasome pathway. This releases and activates NF-κB, which is translocated to the nucleus and binds to DNA for the transcription of inflammatory proteins. Activation of NF-κB by receptor-activated nuclear factor-κB ligand in monocytes may promote bone resorption through cytokine production. In short, NF-κB is a major molecule promoting inflammation in periodontitis.

AP-1 is a two-gene-dependent transcription factor (Jun and Fos), and is capable of producing some cytokines (e.g., IL-8) and MMPs involved in periodontitis. The monomers (c-Jun, c-Fos, v-Jun, v-Fos, Fos, Fra-1, Fra-2, Jun-D, Jun-B, and ATF) can generate a homodimeric complex (Jun/Jun) or a heterodimeric complex (Jun/Fos). This transcription factor family is critical to the early genetic regulation of immune responses. AP-1 is activated by another specific MAP kinase, c-Jun *N*-terminal kinase (JNK). Similar to IKK, JNK phosphorylates c-Jun, following TNF-α-receptor stimulation, thereby inducing AP-1 activation.

All of the mediators and proteins mentioned above are involved in periodontal diseases, and their physiologic inhibition seems to be crucial to periodontal tissue turnover, and to triggering the processes of regeneration.[52] In contrast to H_2O_2, HOCl does not significantly activate JNK, except at a lethal dose (50 μM), thus preventing the tissue destruction caused by MMPs and IL-8. The extremely low doses (20–50 μM) of HOCl required for MAP kinase activation, compared with H_2O_2 (400–1000 μM), may be due to the high reactivity of HOCl with thiol groups and not to physiologic enzymatic degradation.[53]

A toxic concentration of HOCl oxidizes and causes irreversible loss of intracellular protein thiol groups, including glutathione, glutaredoxin and thioredoxin, resulting in their cross-linking and aggregation.[54] Low doses of HOCl oxidize preferentially accessible thiol residues of cysteine amino acids of vital cellular antioxidants, such as reduced glutathione, thioredoxin and glutaredoxin.

Thioredoxin expression modulates NF-κB activity at three levels. In the nucleus, it helps NF-κB bind to DNA.[55] In the cytosol, it activates NF-κB at a downstream level of NF-κB-inducing kinase;[56] while near cell membranes, it inhibits NF-κB-mediated cytokine production at a level upstream of NF-κB-inducing kinase and at a level downstream of TNF receptor-associated factor protein.[57] HOCl may oxidize cytoplasmic antioxidants close to the cell membrane, and thioredoxin may thus be unable to inhibit NF-κB-mediated cytokine production in this case. Therefore, HOCl here increases inflammatory mediator release.

In contrast to thioredoxin, glutaredoxin expression increases NF-κB activation and, like thioredoxin, increases AP-1 activation. HOCl oxidation in these cases seems to be anti-inflammatory.

Nonspecific oxidants, like H_2O_2 and HOCl, may modulate IκB kinase activities, which then induces phosphorylation of the tyrosine residue 42 instead of the serine residues 32/36 of IκB-α, triggering the dissociation of IκB-α from the NF-κB dimer. Interestingly, IκB-α dissociates without degradation by the 26S proteasome mentioned above.[58] In conclusion, HOCl may indirectly regulate these transcription factors and inflammation.

Thus, although HOCl has the ability to inhibit redox-sensitive transcription factors, similarly to TauCl, antioxidant-mediated HOCl neutralization prevents this activity *in vivo*. In fact, moderate HOCl-induced depletion of antioxidants may favor HOCl-mediated nonspecific activation of protein tyrosine kinases, i.e., MAP kinases, which leads to nonspecific proinflammatory gene transcription. Therefore, with the mechanism affecting α_2-macroglobulins, nontoxic HOCl concentrations induce cell proliferation and stimulate ECM component production in human fibroblasts.[59]

Influence of TauCl, which scavenges production of HOCl, in periodontal disease

TauCl, a product of the neutrophil myeloperoxidase-halide system, formed by a reaction of taurine with

HOCl, is known to be a long-lived antimicrobial and anti-inflammatory oxidant.[60] As it can be formed by HOCl, it reveals some of the same properties. Nevertheless, it has more potent anti-inflammatory effects.

TauCl exerts a direct concentration-dependent inactivation of type VII collagenases,[43,44] inactivates α_1-proteinase inhibitors[46,47] like HOCl, reduces the HOCl-mediated increase in vascular permeability,[49] significantly inhibits the *in vitro* cell production of various inflammatory mediators and ROS (e.g., IL-1β, IL-6, and IL-8) in LPS-stimulated human adherent monocytes, inhibits lymphocyte proliferation,[61] interferes with transduction signals which generate MMP-9 expression in LPS-stimulated murine peritoneal macrophages,[62] inactivates NF-κB by reducing the translocation of NF-κB and its DNA-binding activities, inhibits the production of MCP-1, MIP-2, IL-1β, IL-2, IL-6, IL-8, TNF-α, NO, and prostaglandin E_2 due to the involvement of the NF-κB and AP-1 transcriptional pathways,[34,63] inhibits the NF-κB-related transcription of inducible nitric oxide synthase and TNF-α genes in a rat model of bronchoalveolar macrophages,[64] and inducible nitric oxide synthase, cyclooxygenase-2, TNF-α, MCP-1 and MIP-2 genes in rat cortical astrocytes,[63] and inhibits cyclooxygenase-2 gene NF-κB post-transcriptional events which are more accessible than transcription, implying that the inhibition is mainly at the post-transcriptional level.[65] Similarly, TauCl essentially suppresses the translation of TNF-α mRNA[61] and induces inhibition of IKK activity by maintaining unphosphorylated cytoplasmic IκB-α. TauCl reduces IKK activation at a downstream rather than an upstream level in the kinase cascade. Instead of serine 32/36 phosphorylation, TauCl induces oxidation of IκB-α methionine 45, yielding a sulfoxide residue. This oxidation is likely to induce a spatial structural change that masks serine 32/36, preventing phosphorylation, or avoids phosphorylated IκB-α recognition by F-box proteins and subsequent lysine 21/22 ubiquitination.[66] Consequently, it inhibits the activation of NF-κB and degradation of IκB-α. The above information is summarized in Table 1.

Therefore, TauCl has the ability to inhibit the production of the principal inflammatory mediators involved in the pathogenesis of periodontitis. These inhibitions may involve activities at the level of gene transcription, at the post-transcriptional stage, and/or at mRNA translation. Consequently, TauCl not only protects tissues against excess HOCl (an antioxidant effect), but also possesses better anti-inflammatory and healing promotion properties.

The successful wound healing performance of HOCl was proven by Selkon et al.,[67] who evaluated the effect of HOCl in treating chronic venous leg ulcers. They determined that using HOCl washes as an adjunctive therapy for recalcitrant venous leg ulcers appreciably increased healing and rapidly relieved pain. HOCl washes were given over 12 weeks to patients who failed to achieve a 44% ulcer reduction after 3 weeks of standard treatment. Encouraging results were shown after 3 weeks in which 45% of 20 ulcers had healed and a further 25% were reduced by over 60% in size.

However, adversely, HOCl did not promote wound healing compared with electrolyzed water in another study.[68] The author also quoted from the study of Kozol et al. that sodium hypochlorite (NaOCl) had toxic effects on wound healing (e.g., on neutrophils, fibroblasts, and endothelial cells even at dilute concentrations of $0.0005-0.025\%$).[69] Wounds created on mouse ears and treated with 0.25% NaOCl showed delayed epithelialization and neovascularization.[70] The materials used in those experiments were not pure acidic HOCl, but alkaline NaOCl or HOCl in a saline solution. Thus, one cannot conclude that HOCl is detrimental to healing based on their conclusions.

Stabilized, healing-promoting HOCl should be kept at a pH of $3.5-5$ in order to maintain its desired activity.[71] HOCl underwent antibacterial and wound healing tests, and it was found that the minimal bactericidal concentration of HOCl for most bacteria used in those experiments was $<3\,\mu g/mL$ at room temperature for 60 minutes, except for *Aspergillus niger*, which required $86.6\,\mu g/mL$. The time required for bacteria to be killed was the least (within 1 minute), and it had the greatest relative therapeutic index when OCl^- was compared with H_2O_2. In the wound healing test, healing was more obvious after the use of HOCl than saline, and atraumatic wiping with HOCl between dressing applications seemed to favor healing. Although in that research, specific periodontal pathogens were not included; it still indicated the importance of HOCl in wound healing and the antibacterial properties of HOCl.

Conclusion

Excessive ROS seem to be harmful to human tissues, while HOCl is able to modulate the inflammatory response, often in a concentration-dependent manner, and may have both proinflammatory and anti-inflammatory characteristics. The anti-inflammatory effects would appear to predominate, but the outcomes of these multiple effects *in vivo* require further exploration. TauCl, however, possesses mostly anti-inflammatory properties, and may promote healing and alleviate inflammation.

Based on the above viewpoints, the Periodontal Department of Taipei Medical University Hospital began using an ultrasonic spray of HOCl (HSP-600SME;

Table 1. Summary of extracellular effects of hypochlorous acid (HOCl) and taurine chloramine (TauCl)

	HOCl			TauCl*	
	Effects	Inflammation-enhancing	Healing-promoting	Effects	Healing-promoting
Type VII collagenases	High concentration: inhibition Low concentration: promotion	↓	↑	Direct concentration: dependent inactivation	↑
Activation of the proform of matrix metalloproteinase 2, collagenase 2, and gelatinase	High concentration: inhibition Low concentration: promotion	↓	↑	Not stated	
Increase of vascular permeability	Present	↑		Inhibit HOCl induction of vascular permeability	
Neutralization of proinflammatory cytokines and chemokines	Present		↓	Present	↓
NF-κB activation	Present (due to oxidation of thioredoxin and phosphorylation of IκB-α) Inhibit (due to oxidation of glutaredoxin)	↑	↓	Inhibit	↓
IκB kinase activation	Not stated			Inhibit	↓
Mitogen-activated protein activation	Extremely low dose	↑		Not stated	
Activator protein 1 activation	Inhibit		↓	Inhibit	↓
c-Jun *N*-terminal kinase activation	No, except lethal dose		↓	Not stated	
α_2-Macroglobulin oxidation (release of free growth factor)	Present		↑	Not stated	
Activation of TGF-β	Present		↑	Not stated	

*TauCl seems to promote the healing of periodontitis. ↑ = promotes; ↓ = inhibits.

Biotech Corp., Taiwan) for constant sterilization and infection control of clinical cubicles and wound irrigation during periodontal surgery in 2007. Application of HOCl through an ultrasonic delivery system may prove to be a good modality for preventing infections in hospitals.

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