Hydrogen Peroxide: A Review of Its Use in Dentistry

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SEVERAL DENTIFRICES THAT CONTAIN HYDROGEN PEROXIDE are currently being marketed. The increased use of bleaching agents containing (or generating) H_2O_2 prompted this review of the safety of H_2O_2 when used in oral hygiene. Daily exposure to the low levels of H_2O_2 present in dentifrices is much lower than that of bleaching agents that contain or produce high levels of H_2O_2 for an extended period of time. Hydrogen peroxide has been used in dentistry alone or in combination with salts for over 70 years. Studies in which 3% H_2O_2 or less were used daily for up to 6 years showed occasional transitory irritant effects only in a small number of subjects with preexisting ulceration, or when high levels of salt solutions were concurrently administered. In contrast, bleaching agents that employ or generate high levels of H_2O_2 or organic peroxides can produce localized oral toxicity following sustained exposure if mishandled. Potential health concerns related to prolonged hydrogen peroxide use have been raised, based on animal studies. From a single study using the hamster cheek pouch model, 30% H_2O_2 was referred to as a cocarcinogen in the oral mucosa. This (and later) studies have shown that at 3% or less, no cocarcinogenic activity or adverse effects were observed in the hamster cheek pouch following lengthy exposure to H_2O_2. In patients, prolonged use of hydrogen peroxide decreased plaque and gingivitis indices. However, therapeutic delivery of H_2O_2 to prevent periodontal disease required mechanical access to subgingival pockets. Furthermore, wound healing following gingival surgery was enhanced due to the antimicrobial effects of topically administered hydrogen peroxide. For most subjects, beneficial effects were seen with H_2O_2 levels above 1%. *J Periodontol 1995;66:786–796.*

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In dentistry, 3% hydrogen peroxide has been used primarily to enhance recovery from gingival surgery and to reduce plaque as well as levels of microbial organisms involved in periodontal disease. Levels of H_2O_2 or H_2O_2-generating systems used for cosmetic purposes, generally bleaching, deliver > 3% H_2O_2. This review will focus on routine use of low levels (3% or less) of H_2O_2 in oral hygiene, as well as use of high levels required for tooth bleaching. Major concerns raised by long-term use of H_2O_2 include potential irritation, yeast overgrowth, and carcinogenic enhancement. This review will address potential mechanisms of action, in vitro studies, and in vivo animal studies, as well as routine and therapeutic dental usage.

Background

The use of hydrogen peroxide to decrease plaque formation and control "pyorrhea" was first reported in 1913.[1] Sodium bicarbonate has also been used to treat periodontal inflammation since the early 1900s.[2] Hydrogen peroxide can exhibit antimicrobial effects through release of oxygen, and pathogenic effects are seen in Gram-positive as well as Gram-negative organisms.[3] Several factors are necessary for the antimicrobial effects of hydrogen peroxide to occur. Concentration and length of exposure are most important, but the presence of organic and inorganic materials also influences the efficacy of this agent.

The efficacy of hydrogen peroxide is enhanced by the presence of trace metals such as iron and copper, which accelerate decomposition of hydrogen peroxide to hydroxyl radicals from the following reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH- + \bullet OH \text{ (hydroxyl radical)}$$

The overall effect of combining hydrogen peroxide with iron is rapid decomposition of hydrogen peroxide with the intermediate formation of reactive oxygen spe-

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cies as indicated above. Ultimately, oxygen and water are formed from the interaction of H_2O_2 and Fe:

$$2\ H_2O_2 + \text{Fe salt} \rightarrow 2\ H_2O + O_2$$

Hydrogen peroxide interacts with Cu^{1+} salts to a greater extent than Fe^{2+} to form hydroxyl radical intermediates.

Hydrogen peroxide is one of several types of reactive oxygen species produced by the body's cellular defense system that are active against invading microorganisms.[4,5] These oxygen species are reactive and short-lived, which precludes certain species from crossing cell membranes. In addition to hydrogen peroxide, superoxide is also produced by tumor cells[6] and phagocytes[7] as part of the body's cellular defense mechanism. Superoxide can also interact with hydrogen peroxide to form hydroxyl radicals.

The role of hydrogen peroxide as an antimicrobial agent has been firmly established and as a 3% aqueous solution, it is widely used as a disinfectant. One mechanism of antibacterial action is the liberation of oxygen following decomposition by protective enzymes such as catalase. Because oxygen is toxic to anaerobic organisms, survival is decreased in the presence of oxygen. There is evidence that in the presence of metals, hydrogen peroxide causes breaks in DNA strands,[8] an effect also seen with ionizing radiation.[9] Activation of intracellular water molecules by ionizing radiation can result in formation of hydroxyl radicals as well as hydrogen peroxide. When intracellular Fe^{2+} or Cu^{1+} are present, hydrogen peroxide can react with them to form hydroxyl radicals that can potentially react with cellular nucleophiles. Gene loss or mutation can result from breaks in DNA strands caused by these highly reactive oxygen radical species.

Strand breakage exposes more DNA due to relaxation of the protective histone binding. Through DNA unwinding, exposure of additional sites for electrophilic attack near strand breaks can result in greater damage from other reactive oxygen species or from exogenous agents[10] unless the affected DNA is rapidly and accurately repaired. Chromosome damage by iron and H_2O_2 is enhanced by histidine, which complexes with iron.[11,12] Because histidine can complex with iron and enhance DNA strand breakage by H_2O_2, results from tissue culture systems that use medium containing this amino acid to study cytotoxic and clastogenic effects of H_2O_2 should be interpreted with caution.

H_2O_2 is genotoxic in an oxidant-sensitive tester strain, TA102, of *Salmonella typhimurium.*[13] In contrast, an H_2O_2-generating tooth bleaching agent was not genotoxic in tester strain TA102 or in mammalian cells.[14] Furthermore, administration of 0.5 to 2 g of a bleaching agent or 70 mg/kg H_2O_2 per day for up to 6 months did not increase bone marrow sister chromatid exchange in Chinese hamsters.[15] Genotoxicity was also not seen in tester strain TA102 with up to 267 mM H_2O_2 in a dentifrice containing H_2O_2 incorporated into a polaxomer gel.[16] Optimal mutagenicity for aqueous H_2O_2 was achieved in tester strain TA102 at 1.34 mM. Oxidant-trapping agents such as sorbitol that are present in the product formulations could explain the lack of activity by the dentifrice and tooth bleaching agent.

The mechanism of hydrogen peroxide-induced cytotoxicity in mammalian cells has not been determined. Cytotoxicity may occur from direct effects on cell membranes that can lead to cell death through loss of membrane integrity, and by inactivation of critical cell components once the cell membrane has been breached and cellular defense mechanisms have been exhausted.[17]

Both intra- and extracellular defense mechanisms are present to protect cells from reactive oxygen species. In the oral cavity, a salivary peroxidase system can provide extracellular detoxification of hydrogen peroxide.[18] A peroxidase is also present in plasma to prevent damage from reactive oxygen species that enter the circulatory system.[19] Intracellularly, hydrogen peroxide is converted to water and oxygen by glutathione peroxidase. Catalase also breaks down hydrogen peroxide to oxygen and water. Likewise, superoxide dismutase breaks down superoxide. Controlled delivery of reactive oxygen species, as in antibiotic therapy, presents a special challenge due to the extreme reactivity of oxygen radical species, the presence of cellular detoxification systems, and the potential for cellular damage at high levels.

REACTIVE OXYGEN SPECIES AND DISEASE

Inflammation

Chronic inflammation subjects nearby cells to elevated levels of reactive oxygen species due to extracellular release from phagocytic cells.[20,21] H_2O_2 secretion also triggers recruitment of additional phagocytes, which further exacerbates the inflammatory response. Some tumor cells are resistant to H_2O_2 and can tolerate levels up to 10 mM. Consequently, harmful effects of the immune surveillance system on oxidant-resistant cells are diminished.[7] Stimulation of neutrophils with 12-O-tetradecanoyl phorbol-13-acetate (TPA) caused cocultured gingival epithelial cell detachment.[22] Epithelial cell lysis by TPA-stimulated neutrophils was attributed to the myeloperoxidase system, and detachment to H_2O_2 or proteases. Thus, PMNs may play a significant role in gingivitis and periodontitis through release of reactive oxygen species.

Reactive oxygen species released by neutrophils stimulated by TPA were reported to cause malignant transformation of mammalian cells[23] and mutation of bacteria.[24] It has been estimated that TPA-stimulated neutrophils produce 1.4 nmol $H_2O_2/10^4$ cells/h and unstimulated tumor cells produce H_2O_2 at half this rate.[7] During exposure to opsonized zymosan, neutrophils released 4.7 nmol $H_2O_2/10^6$ cells during the first 15 minutes of exposure, compared to 1.2 nmol $H_2O_2/10^6$ alveolar macrophages and 0.5 nmol $H_2O_2/10^6$ monocytes during the same

time period; production of superoxide was 2 to 4 fold greater.[25] Thus, significant levels of H_2O_2 and other reactive oxygen species can be released locally by phagocytes, which may prove to be a significant factor in gingivitis. Furthermore, as previously mentioned, superoxide can interact with H_2O_2 to produce hydroxyl radicals.

Constant exposure to reactive oxygen species may also enhance damage in affected cells by exogenous chemicals.[10,26,27] Of particular interest in mammalian cells is the activation of c-*fos* and c-*jun,* two genes involved in growth stimulation, by low levels of H_2O_2, up to 250 μM.[28,29] Thus, a coordinated sequence of events: breakage of DNA strands and repair; signal transduction; and induction of transcriptionally-active enzymes can occur that can result in growth stimulation following exposure to low levels of H_2O_2. Other genes can be activated that lead cells on a pathway toward programmed cell death, or apoptosis.[30]

Cancer

Because of the propensity for H_2O_2 and other reactive oxygen species to damage DNA, major concerns from long-term hydrogen peroxide exposure are tumor development or enhancement of tumor formation. The only reports of complete carcinogenicity of hydrogen peroxide have come from Ito and coworkers.[31,32] In these studies, gastric lesions were observed in mice after 10 weeks exposure to 0.4% H_2O_2 in their drinking water. The total dose of H_2O_2 consumed during this period was 150 to 200 μg. No lesions of the oral mucosa were reported in animals from these studies. It is important to note, however, that statistical significance for tumor induction was only achieved by combining the data from males and females. When analyzed separately, there were no significant differences between control and H_2O_2-treated groups. Because of sex-related differences in responses to xenobiotics, it is recommended that sex-related statistical analyses be performed.

Development of tumors, described as hyperplastic lesions, varied among strains of mice exposed to H_2O_2, and the extent of tumor induction was negatively correlated with catalase activity which also differed among strains of mice.[33] Similar results (complete carcinogenicity) have not been reported for hydrogen peroxide by other laboratories or in other animal models. The lesions observed in the Ito studies were mainly preneoplastic (hyperplasia) that can revert to normal following removal of the stimulus. Of particular interest was the observation that lesions were more severe in the duodenum than in the gastric mucosa. The duodenum reportedly has the lowest organ catalase activity in C57BL/6 mice,[33] and the spontaneous incidence of duodenal plaques has been reported to be as high as 70% in untreated female C57BL/6 mice.[34,35] The reversibility of lesions induced in C57BL/6 mice by H_2O_2 was also demonstrated by replacement of drinking water containing 0.4% H_2O_2 with distilled water for 10 to 30 days.[31] Thus, the complete carcinogenicity of H_2O_2 has not been demonstrated using standard bioassay and analytical techniques. Although acatalasemic individuals have been identified, other antioxidant defense mechanisms apparently compensate for the catalase deficiency.[36] Therefore, using results obtained in C57BL mice in human risk assessment is of questionable value.

Tumor Promotion

Tumor promotion (enhanced tumor incidence) can occur following multiple administrations of a compound subsequent to a subthreshold dose of a known carcinogen. Characteristics of tumor promoters are a lack of carcinogenicity when administered alone and a requirement for multiple exposures. Administration of a tumor promoter prior to an initiator will not result in tumor formation.[37] Cerutti and Trump and Cerutti have suggested that continuous exposure to reactive oxygen species can result in a prooxidant condition that renders tissue more susceptible to disease.[26,27] There is evidence from Viaje and coworkers to support this hypothesis in mouse skin where the most potent tumor promoters are those that invoke inflammatory responses. Furthermore, a reduction in tumor yield can be obtained in this animal model with anti-inflammatory agents.[38]

Benzoyl peroxide, an organic hydroperoxide, is an effective tumor promoter in mouse skin.[39] In contrast, 30% hydrogen peroxide showed very weak tumor promoting activity, with a 6% papilloma incidence (0.06 papillomas/mouse) when administered twice weekly for 25 weeks;[40] the papilloma incidence was 10% (0.15 papillomas per mouse) from twice weekly applications of 6% H_2O_2. In contrast, TPA produced a tumor incidence of 94% (9.9 papillomas per mouse) at 2 μg/mouse when administered twice weekly for 13 weeks.[41] The maximum amount of H_2O_2 administered was 600 mg, compared to 52 μg TPA. Based on the DMBA doses administered, only 12% of the skin papillomas would progress to carcinomas after 60 weeks,[41] with an overall tumor incidence of 0.018 carcinomas per mouse in animals given DMBA and H_2O_2. In contrast, the overall tumor incidence for DMBA and TPA would be 1.19 carcinomas per mouse, or nearly 100 times greater than that expected from H_2O_2 at a dose of TPA that was more than 1,000 times lower than H_2O_2. In another study, ICR mice did not develop skin tumors after 58 weeks following initiation with DMBA and promotion with 3% H_2O_2.[42] Given the weak tumor promoting effects of 6% and 30% H_2O_2, it is not surprising that 3% H_2O_2 is inactive as a tumor promoter. In the same study, tumor promoting activity also was not observed with 5% urea peroxide (carbamide peroxide).

In other animal tumor models, such as the development of squamous cell carcinomas in hamster cheek pouches following DMBA administration, an inflammatory re-

sponse had mixed effects on tumor yield. Some authors reported an enhanced tumor yield with irritation,[43] while others reported the opposite effect.[44] Because age-related differences in tumor response were reported, the stage of development may play a significant role in stimulation or inhibition by inflammatory agents. According to Silberman and Shklar,[44] croton oil was an effective cocarcinogen in hamsters 12 months of age. Hamsters in this age group had a lower inflammatory response than did animals 2 to 3 months of age. Because the incidence of adverse health effects unrelated to treatment is greater in older animals and increased carcinogen susceptibility is usually seen in young animals, hamsters 2 to 3 months of age are generally used for carcinogenesis studies. However, they may not be susceptible to the cocarcinogenic effects of croton oil. The hamster cheek pouch may also be more resistant to cocarcinogenic or tumor promoting agents, such as phorbol esters, that are active on epidermis.

Although tumor promoting activity by croton oil has been reported in hamster cheek pouches following DMBA administration,[45] other investigators did not observe a tumor promoting effect with croton oil following administration of arecaidine, a carcinogen found in betel nuts.[46] The component of croton oil that produces the greatest tumor promoting response in mouse skin is TPA. In the hamster cheek pouch, TPA initially produces a hyperplastic response that is diminished by subsequent exposure.[47,48] Lack of epithelial thickening (a premalignant response) in hamster cheek pouch by TPA was also reported by Gimenez-Conti and Slaga.[49] These observations indicate that results obtained in mouse skin may not be applicable to other tissues, particularly one so morphologically different as the oral mucosa of the hamster cheek pouch. In contrast to TPA, benzoyl peroxide has tumor promoting activity in this animal tumor model.[50,51] Significant differences exist between H_2O_2 which can readily pass through cell membranes and hydrophobic organic hydroperoxides such as benzoyl peroxide. Because the primary site of action of organic hydroperoxides is the cell membrane, a cascade of radical-mediated lipid peroxidation events can occur that result in co-oxidation of hydrophobic carcinogens.[52]

Other agents with tumor promoting activity in the hamster cheek pouch tumor model include noncarcinogenic doses of DMBA,[53] ethanol,[54] mechanical irritation,[55] and X-radiation.[56] In all of these studies, DMBA was used as an initiator. Padma and coworkers[57] have also reported tumor promoting activity by H_2O_2 in hamster cheek pouches following initiation with the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Although distal tumor incidence was increased in animals receiving NNK and H_2O_2, there was no increase in tumors at the site of application. Furthermore, the endpoint lesions were preneoplastic rather than malignant tumors. In these treated hamsters, 1/8 animals (12.5%) had papillary hyperplasia of the cheek pouch after 14 months; 0/9 had papillary hyperplasia by 22 months.[57]

In a tumor promotion regimen in which a single 10 mg dose of NNK was administered, followed by a total dose of 2400 μL 30% H_2O_2 (720 mg H_2O_2: 20 μL × 120 applications, 5 × weekly), papillary hyperplasia in cheek pouches was 1/23 (4.3%) at 14 months. For short-term NNK administration (10 mg) followed by 30% H_2O_2, forestomach papilloma incidence was 6/23 (26.1%) after 14 months. Lung adenomas and hepatomas were also increased in animals receiving NNK and 30% H_2O_2, compared to NNK alone. Although it is difficult to interpret the results obtained in lung and liver (the major target organs for NNK), in the cheek pouch (point of application) and forestomach, only hyperplasia was observed. Because of high levels of glutathione peroxidase and catalase in liver, lung and the gastrointestinal system, very low levels of H_2O_2 would be expected to survive transport to the liver.

Cocarcinogenic Activity

A compound is considered to be a cocarcinogen if it is administered concurrently with a low dose of a known carcinogen and the resulting tumor incidence is greater than that of the carcinogen alone. In the aforementioned hamster study with NNK and 30% H_2O_2,[57] papillomas of the forestomach were observed in hamsters receiving simultaneous administration of NNK and 30% H_2O_2 (cocarcinogenesis treatment protocol) for 14 months, and the forestomach tumor incidence was 7/18 (38.8%), compared to 2/8 (25%) for NNK alone. No information was provided as to possible direct interactions between NNK and H_2O_2 during the cocarcinogenesis phase of this study.

Weitzman et al.[58] reported that hydrogen peroxide was cocarcinogenic in the hamster cheek pouch model when administered on alternate days with 0.2% DMBA. Data from this study are of marginal statistical significance because of the small number of animals utilized and the weak effect observed with 30% H_2O_2. Furthermore, no differences were observed between control and experimental groups after 19 weeks; after 22 weeks the 3% H_2O_2 treatment group tumor incidence was no different than that of the group treated with 0.2% DMBA alone.

Dentifrices formulated to deliver 0.75% H_2O_2, 1.5% H_2O_2, or a 1:1 mixture of 3% H_2O_2 and sodium bicarbonate, did not show any evidence of cocarcinogenicity after 20 weeks when administered concurrently with DMBA for 16 weeks or 20 weeks.[59] In fact, time-to-tumor analysis indicated a significant increase in tumor latency when 3% H_2O_2 and sodium bicarbonate were applied concurrently with 0.25% DMBA, compared to 0.25% DMBA alone.[59] With 0.25% DMBA, tumor latency was unaffected by a dentifrice containing 1.5% H_2O_2. These results

are consistent with previous reports[58] that showed no enhancement of DMBA-induced carcinogenesis by 3% H_2O_2.

HYDROGEN PEROXIDE IN BLEACHING AGENTS

The safety and efficacy of H_2O_2 and carbamide peroxide use in vital tooth bleaching have recently been reviewed.[60–62] Although bleaching agents containing H_2O_2 or carbamide peroxide are available over the counter, tooth bleaching is best performed under dental supervision, where higher levels can be administered to achieve better results, and the bleaching process can be closely monitored.

Formulation

For tooth bleaching, H_2O_2 is used alone at levels up to 35%, at 3 to 10% levels in a stable gel, or generated from a stable gel of 10 to 15% carbamide peroxide (urea peroxide) that breaks down into urea, ammonia, carbon dioxide, and hydrogen peroxide (3.35% H_2O_2 from 10% carbamide peroxide). The release of hydrogen peroxide from carbamide peroxide can be prolonged by the addition of carboxypolymethylene polymer.

Acute Toxic Effects of Hydrogen Peroxide

The oral LD_{50} of H_2O_2 (9.6 to 60% solutions) in rats was reported to be between 801 and > 5,000 mg/kg for single administrations.[63] In mice, the LD_{50} of H_2O_2 (90%) was reported to be 2,000 mg/kg for a single administration.[63] When H_2O_2 was administered in the drinking water for 210 days, the lowest observed effect level in mice (glandular stomach lesions) was found at 0.1% H_2O_2.[63] Ingestion of greater than 10% hydrogen peroxide can be deleterious, and fatalities have been reported in children ingesting 27% and 40% H_2O_2.[64] In the same report, an infant survived without complications after ingesting a mouthful of 35% H_2O_2 once aggressive treatment was initiated. The major concerns following concentrated H_2O_2 ingestion are necrosis, stomach rupture from liberated oxygen, and mechanical asphyxia from obstruction of the distal respiratory tract because of foaming.

Carbamide Peroxide and Bleaching

Although the use of peroxides in tooth bleaching agents has recently been reviewed,[60–62,65,66] and a large body of data exists for use of hydrogen peroxide, long-term studies of carbamide peroxide use are lacking. Tooth bleaching requires prolonged contact between the bleaching agent and the dentin surface, and the bleaching process is accelerated by heat. A barrier system is also required to protect the sensitive gingival tissue from the effects of prolonged peroxide exposure. There is also evidence that toxic peroxide levels can be attained in the pulp region of teeth exposed to bleaching agents.[67–69] Gingival dehydration may also occur from exposure to the anhydrous glycerin base.

Some carbamide peroxide bleaching systems contain acid to accelerate the bleaching process, but the presence of acid is destructive to tooth enamel. In contrast, hydrogen peroxide-containing bleaching systems perform well at neutral pH and should be less harmful to gingival tissue. Bleaching systems with H_2O_2 for at-home use and office-monitoring that employ 2 to 10% levels of H_2O_2 also require shorter exposure periods than are required with carbamide peroxide. Release of H_2O_2 can be delayed by incorporation of carboxypolymethylene polymer into the bleaching gel.[60]

Toxic Effects of Carbamide Peroxide

Few data are available on the acute effects of carbamide peroxide. No significant deleterious genotoxic effects were observed in animals or in mammalian cells.[15] In contrast, acute toxicity was seen in rats at 5 mg/kg of a tooth whitener containing 35% carbamide peroxide when given by gavage.[70] A subsequent study indicated that carbamide peroxide-containing products were not genotoxic, nor were they any more toxic than dental materials currently in use.[71] In mice, acute toxicity of carbamide peroxide-containing dental materials was enhanced by carboxypolymethylene polymer, presumably due to extended release of activated oxygen species from carbamide peroxide.

Effects on Enamel

Etching of enamel sufficient to facilitate entry by opportunistic microflora may occur following exposure for 30 hours to high levels of peroxides found in bleaching agents.[72] Softening of the enamel also occurred following a 12-hour exposure to 10% carbamide peroxide gel.[73] No morphologic surface changes, as assessed by electron microscopy, were seen in another study in which carbamide peroxide was placed in contact with teeth for 72 hours.[74] Compared to posterior or hybrid composite resins, a reduction in tensile strength was observed in microfilled composite resins exposed to 30% H_2O_2 at 37°C for one week.[75] Although long-term recovery following bleaching agent exposure was not assessed in this study, removal of the bleaching agent restored opacity and whiteness.

Effects of Bleaching Agents on Pulp and Gingiva

Bleaching agents can enter the pulp via leakage from tooth restorations, particularly at the cemento-enamel junction and following thermal stress.[76] Treatment with 33% H_2O_2 and heat for 120 to 180 minutes (4 treatments of 30 to 45 minutes at 2-week intervals) can result in loss of tooth vitality, presumably from accumulation of toxic levels of H_2O_2 in the pulp.[77] Cytotoxic levels of H_2O_2 can also accumulate inside an in vitro pulp chamber in a relatively short period of time after penetration through dentin.[67–69] After an hour exposure to bleaching agents, mM

concentrations of H_2O_2 could be detected after migration through a 0.5 mm thickness of dentin.[67] These in vitro experiments were performed in the absence of protective enzymes such as catalase and glutathione peroxidase. In vivo, these defense mechanisms would significantly reduce available levels of H_2O_2. In the oral cavity, protective systems include a salivary peroxidase[18] and other antioxidants, including reduced glutathione.[78] An ID_{50} of 0.58 mM was established for H_2O_2 in murine fibroblasts with intact antioxidant defense mechanisms.[67]

Reversible changes in pulp were observed in canine teeth exposed to 35% H_2O_2 for 30 minutes in the presence or absence of heat (62°C) for 30 minutes.[79] Inflammatory responses, odontoblast destruction, and resorption were observed, but these alterations resolved by 60 days post exposure. Enamel and dentin thickness averaged 1.7 mm, which is less than that of humans. Thus, the effects expected in humans would be reduced, because the amount of H_2O_2 reaching the pulp would be less than in the canine study. When ovine dental pulp was exposed to H_2O_2 in the presence or absence of heat (50°C) for up to 30 minutes, enzyme activities were decreased, indicating toxicity can occur if H_2O_2 reaches the pulp.[80]

When 11% carbamide peroxide in anhydrous glycerin gel was used for oral hygiene, plaque levels decreased, but gingival status did not improve.[81] The effect on plaque has been attributed to denaturing effects of urea on the proteinaceous plaque matrix although antimicrobial effects of hydrogen peroxide release should not be discounted. Gingival inflammation was not reduced, presumably as a result of deep periodontal pockets that were not accessible to the hydrogen peroxide, and to subgingival calculus as an irritant.

In another study, three different carbamide peroxide bleaching formulations were compared for efficacy following 3 hours or overnight exposures.[82] With all three formulations, regardless of treatment time, gingival indices improved with treatment. While plaque indices improved, there was no difference between treated and untreated groups. Thus, beneficial effects of reduced plaque formation and gingival inflammation can be seen following carbamide peroxide treatment.

Bleaching Agent Summary

Two types of bleaching systems are generally employed. The strongest bleaching systems are applied only in the dental office to enable careful monitoring. At-home bleaching systems use carbamide peroxide or hydrogen peroxide as the active ingredient. For most home applications, the levels of hydrogen peroxide employed are 2 to 10%. Carbamide peroxide-based systems with 10 to 15% carbamide peroxide release 3 to 5% H_2O_2. Other components in carbamide peroxide-based systems, such as urea, may assist in plaque removal due to protein denaturation. High levels of peroxide can penetrate the dentin and cause toxicity to the pulp. Exposure of the pulp to peroxides may also be facilitated by weakening of restorative materials, particularly at the cemento-enamel junction (CEJ).

ORAL HYGIENE AND LOW LEVELS OF HYDROGEN PEROXIDE

Acute Studies in Animals

Erosion occurred in canine gingiva following continuous exposure to 1% H_2O_2 for up to 48 hours.[83] Localized inflammation and edema occurred within 6 hours, suggesting an effect of H_2O_2 on vascular permeability. An acute localized response (edema) was reported in canine tongues exposed continuously to dilute (0.3 M) H_2O_2 for at least 4 hours.[84] Localized mild inflammatory responses were also observed in patients exposed to 10% carbamide peroxide gel.[69] However, no changes in soft tissue were reported when the same material was applied to hamster cheek pouches.

Short-Term Studies

Oral ulceration has been reported for short-term exposure (as few as three treatments) of 3% H_2O_2 in combination with a hypertonic salt solution.[85] The lesions occurred after a hypertonic saline (5M) oral rinse following brushing with a 3% H_2O_2/sodium bicarbonate mixture as recommended by Keyes.[86] When water was substituted for 5M NaCl as the oral rinse, no adverse gingival effects were observed. These authors concluded that the irritant effect was due to the hypertonic saline rinse rather than H_2O_2. In another report,[87] one individual who was using 3% H_2O_2 as an oral rinse to treat mucosal ulceration, and another who was using the Keyes oral hygiene regimen with 3% H_2O_2, baking soda, and salt had evidence of ulceration that resolved after cessation of treatment. An additional report describes gingival injuries that occurred in three patients using the Keyes oral hygiene regimen.[88] In these case studies, gingival injuries resolved within 1 to 3 weeks after discontinuing the H_2O_2/$NaHCO_3$/salt oral hygiene regimen. These lesions presumably occurred as a consequence of frequent, vigorous brushing with the H_2O_2/$NaHCO_3$/salt mixture. In two cases, treatment was initiated when gingival lesions were already present.

Two groups of individuals with different plaque scores (gingival index, plaque index, and gingival crevicular fluid) were compared following a 3 times daily use of a mouthrinse[§] containing 1.5% H_2O_2 for seven days.[89] No ulceration was reported, and the two groups that used the H_2O_2-containing mouthrinse had significant improvements in plaque scores when compared to control groups that used a mouthrinse without H_2O_2. A longer-term study

[§]Peroxyl, Colgate Oral Pharmaceuticals, Canton, MA.

of a mouthrinse with 1.5% H_2O_2 and 0.05% NaF[‖] used daily for up to 18 months showed no evidence of mucosal lesions in orthodontically-banded subjects.[90] Furthermore, overall gingival health (bleeding tendency and plaque index) was significantly improved in this treated group,[‖] compared to a control group that received a 0.05% NaF-containing mouthrinse.[¶] Although fluoride rinses are effective in reducing demineralization and favor remineralization, they are not effective antimicrobial agents. In contrast, there are numerous reports of the antimicrobial activity of H_2O_2. The effectiveness of H_2O_2 in treating gingival disease has been ascribed to a physical effect on plaque removal by the bubbling of oxygen as it is released from the peroxide, a direct antimicrobial effect of H_2O_2, and enhanced wound healing due to the presence of oxygen. In conclusion, Boyd[90] suggested that additional studies be performed to establish the optimal H_2O_2 concentration for effective treatment of gingivitis.

Antibacterial Activity of Hydrogen Peroxide and Sodium Bicarbonate

Hydrogen peroxide is toxic to bacteria associated with periodontal disease, and a synergistic effect can be obtained by combining H_2O_2 with sodium bicarbonate.[91] Bacteria that are susceptible to H_2O_2 alone or in combination with sodium bicarbonate include *Actinobacillus actinomycetemcomitans, Haemophilus aphrophilus, Eikenella corrodens, Capnocytophaga gingivalis, Mycoplasma salivarium, Actinomyces naeslundii, Actinomyces viscosus, Streptococcus salivarius,* and *Streptococcus mutans.* Of these microorganisms, H_2O_2 is more effective in vitro against periodontal disease-causing organisms than cariogenic organisms such as *Streptococcus mutans* and *Streptococcus salivarius.*[92] From these studies, no correlation was observed between bacterial catalase activity and antibacterial activity of H_2O_2. In combination with H_2O_2, low levels of $NaHCO_3$ stimulated microbial growth and increased resistance to oxidant-induced injury until lethal levels of $NaHCO_3$ were obtained. At higher levels, $NaHCO_3$ also acted synergistically with H_2O_2. One possible explanation for the synergistic effect is that $NaHCO_3$ can disrupt the cell membrane of Gram-negative organisms, possibly through a hypertonic effect to facilitate entry of H_2O_2.[93] Thus, higher levels of $NaHCO_3$ enable lower levels of H_2O_2 to be used to achieve the same extent of antimicrobial activity.

Delivery of antibacterial agents to gingival pockets colonized by anaerobic organisms in patients also presents a challenge. When compared with 0.12% chlorhexidine digluconate, 1% H_2O_2 was much less effective in reducing plaque and gingivitis scores.[94] Other clinical studies on delivery of H_2O_2 for treatment of periodontal disease concluded that mouthrinses would not reach deep pockets, and brushing as a means of delivery was also not efficacious.[2] The H_2O_2 concentration employed (1%) may also have been ineffective, as 1.5% H_2O_2 was successful in reducing gingivitis in orthodontic patients.[90] However, no adverse effects were reported from use of a mouthrinse containing 1% H_2O_2 twice daily for 21 days.[94] In a longer study, a mixture of 3% H_2O_2, $NaHCO_3$, and NaCl was placed into subgingival pockets during scaling and root planing, and the patients used this mixture instead of a commercial dentifrice for up to 3 months.[95] Betadine was also applied at the end of professional cleaning which was performed weekly for 4 weeks. In this study, subgingival scaling produced the greatest effect on gingivitis, even when the subjects continued with personal applications of the H_2O_2/$NaHCO_3$/NaCl mixture. A reduction in recoverable spirochetes continued as long as the subjects were enrolled in the study and continued topical treatment. Wound healing was also accelerated in the H_2O_2-treated group. Overall, the beneficial effects of subgingival scaling and root planing were enhanced by subgingival application of H_2O_2/$NaHCO_3$/NaCl, followed by iodine irrigation. Use of antimicrobial agents alone was not as effective as scaling and root planing alone.

Intrasulcular sonication also helped to disperse H_2O_2 as measured by an increase in gingival fluid myeloperoxidase activity.[96] In this study, 0.03% H_2O_2 was delivered with an ultrasonic cavitator, and the low level of H_2O_2 utilized may have provided an indirect antibacterial effect through phagocyte recruitment. Biweekly subgingival irrigation of 3% H_2O_2 for up to 6 months suppressed or completely removed *Actinobacillus actinomycetemcomitans* from periodontal pockets.[97] Again, no adverse effects on gingiva were noted following biweekly application of 3% H_2O_2 for up to 6 months. Three important conclusions can be drawn from these studies: 1) H_2O_2 is effective in reducing plaque; 2) to be effective in reducing periodontal disease, hydrogen peroxide must be delivered to deep pockets; and 3) to reduce gingivitis, a concentration of H_2O_2 sufficient to provide antibacterial activity must be employed.

Clinical Studies of Long-Term Use of Hydrogen Peroxide and Sodium Bicarbonate

Several long-term studies on the efficacy of the Keyes technique for treating gingivitis[98-100] have been performed. Other than the studies previously mentioned in which H_2O_2 was used alone or with NaF as a mouthrinse, most long-term clinical studies have used H_2O_2 in combination with sodium bicarbonate as a treatment for gingivitis.[2] Treatment of gingivitis with sodium bicarbonate and hydrogen peroxide is only part of a regimen that involves professional scaling and root planing, as well as monitoring oral microbial flora.[98] The sodium bicarbonate/peroxide treatment can be performed at home on a daily

[‖]Orthofluoro, Colgate-Hoyt, Norwood, MA.
[¶]Flurigard, Colgate-Hoyt, Norwood, MA.

basis to suppress bacterial growth in the oral cavity. As previously mentioned, toothbrushing alone does not deliver sufficient amounts of this antibacterial mixture to deep gingival pockets containing anaerobic organisms due to the shallow penetration of the bristles within the gingival crevice.[2] Systemic antibiotics may therefore be required to effectively treat gingivitis.

Eighteen patients with moderate to advanced periodontitis were treated by scaling and root planing, or by scaling, root planing, and subgingival pocket elimination.[98] All subjects were treated once daily as recommended by Keyes et al.[86] on one side of their mouths; the other side of each patient's mouth was treated with conventional oral hygiene (brushing and flossing) for 8 weeks after initial scaling and root planing.[98] Plaque and gingival irritation indices decreased throughout the study, regardless of treatment. No significant differences in crevicular fluid flow were observed between treatments. Although improved oral hygiene decreased bacterial flora, the Keyes treatment was no more effective in controlling subgingival microbial flora from deep (> 7 mm) pockets than was conventional hygiene. Approximately 75% of the subjects also reported gingival irritation, which decreased when the amount of H_2O_2 was reduced. These authors concluded that surgical treatment was more effective in producing a favorable clinical outcome and in reducing microbial flora than was topical application of H_2O_2 and sodium bicarbonate as recommended by Keyes. No mention was made of compliance with the treatment regimens in this study other than the reduction in H_2O_2 as previously indicated.

A group of 47 subjects with moderate to severe periodontal lesions participated in a study in which periodontal therapy was guided by microbiological evaluations.[99] Treatment consisted of periodontal pocket irrigation with 1% chloramine-T and salt, daily application of $NaHCO_3$ and H_2O_2 followed by oral irrigation with a salt solution, and systemic administration of tetracycline, as needed to reduce subgingival flora. No control group was included for comparison. Subjects were monitored for 3 to 6.5 years with 2 to 4-month follow-up intervals and subgingival monitoring of microbial flora. Minor gingival irritation was observed initially in some patients, but it resolved when the H_2O_2 level was reduced to 1.5%. No other adverse reactions, such as papillary hyperplasia or yeast overgrowth were recorded. All but one of the subjects also received systemic tetracycline HCl, which had a marked effect on reduction of motile organism and crevicular leukocyte levels. Nineteen subjects received an additional course of tetracycline HCl, and nine subjects required more than one additional course of tetracycline. Significant improvement in periodontal health was observed in all subjects. Careful microbial monitoring during routine follow-up visits enabled additional individualized antimicrobial treatment for each subject. Stabilization or improvement of disease status was noted in all subjects, including ten with refractory periodontal conditions.

A subsequent 4-year study of 171 subjects compared a patient-applied $NaCl/H_2O_2/NaHCO_3$ treatment with conventional oral hygiene.[100] In the conventional oral hygiene arm, 87 subjects used a commercial dentifrice, and 84 subjects used a mixture of 3% H_2O_2 and $NaCHO_3$ instead of a commercial dentifrice. After brushing, gingival areas were irrigated with saturated NaCl or epsom salt. After scaling, root planing, and tooth polishing, subjects returned for evaluation at 8, 16, 24, and 48 months. Compliance was lower in the $NaCl/H_2O_2/NaHCO_3$ treatment arm, compared to the conventional oral hygiene arm. No differences in efficacy were observed between the two study arms. The conclusions were confounded by mechanical intervention (scaling and prophylaxis) when the disease state progressed. There was no indication that subjects who received this intervention were excluded from this study, and this might account for the lack of difference between treatment arms. Furthermore, treatment was performed twice daily in the conventional treatment arm and once daily in the $NaCl/H_2O_2/NaHCO_3$ treatment arm.

Summary of Long-Term Studies

From these and other reports, few if any adverse health effects were seen in large numbers of subjects who used H_2O_2 and sodium bicarbonate on a daily basis for up to 6.5 years. Inflammation was reported in some subjects when saturated salt solutions used for gingival irrigation were included in the treatment regimen. To relieve irritation, the level of H_2O_2 was reduced to 1.5% in early phases of these studies. In the absence of devices to provide access to deep pockets, topical peroxide treatment was ineffective in reducing indices associated with periodontal disease. When used as adjuvant therapy following gingival surgery or subgingival scaling, H_2O_2 and $NaHCO_3$ were effective in reducing the severity of disease, as well as recurrence. No adverse effects were reported from twice daily use of a mouthrinse containing 1.5% H_2O_2. Conversely, few if any benefits were seen with short-term use of hydrogen peroxide and sodium bicarbonate other than a transient decease in microbial flora. It is important to note that efficacy was not associated with use of H_2O_2 at < 1%.

CONCLUSIONS

There is sufficient evidence that H_2O_2 can damage DNA through the intermediate formation of reactive oxygen species, particularly the hydroxyl radical when metals are present. The ability to damage DNA is one factor in the antibacterial activity associated with use of H_2O_2 as a disinfectant. Anaerobic organisms are particularly sensitive to H_2O_2 as molecular oxygen is generated by interaction

with protective enzymes such as catalase. Both intra- and extracellular host defense mechanisms exist to convert reactive oxygen species to molecular oxygen. When combined with sodium bicarbonate, H_2O_2 decomposition is thought to be accelerated,[95] and synergism can occur to decrease levels of H_2O_2 necessary to achieve antibacterial effects. It has been suggested that this synergistic effect may result in bacterial death from membrane damage that facilitates entry of H_2O_2.

Few adverse effects from topical exposure to 3% H_2O_2 have been reported, and the FDA has approved its use as a temporary debriding agent in the oral cavity. While gingival irritation has occasionally been reported with 3% H_2O_2, in most instances irritation occurred when H_2O_2 was administered with sodium bicarbonate followed by a saturated sodium chloride rinse. In all cases, irritation ceased when the percentage of H_2O_2 was decreased. Chronic inflammation has been associated with increased susceptibility to disease due to the presence of reactive oxygen species. Of particular concern are reports of genotoxicity of H_2O_2 in bacterial and mammalian cells. Genotoxicity has not been observed with H_2O_2 formulated in a dentifrice or in bleaching solutions. This result could be explained by the presence of reactive oxygen scavenging agents in the peroxide delivery formulation. Furthermore, H_2O_2 decomposition is accelerated in the presence of sodium bicarbonate.[95]

In animals, enhanced tumor development was reported after exposure to low levels of carcinogen and 30% H_2O_2, although the statistical significance was marginal in some studies. There was no enhancement of the rate of tumor development with 3% H_2O_2. Most tumors seen in these animal models were early preneoplastic lesions, such as papillomas, which can spontaneously regress.

Gingival irritation from bleaching agents, including carbamide peroxide, has been reported. Bleaching agents containing carbamide peroxide must remain in contact with the tooth surface for an extended time to be effective. In contrast, bleaching agents with H_2O_2 can be formulated to reduce acidity, and the effective exposure time can be significantly decreased. Integrity of the enamel is better-maintained with neutral or slightly basic solutions as dissolution occurs under acidic conditions. Furthermore, NaF can be incorporated into solutions of H_2O_2 to promote remineralization.

The antibacterial efficacy of H_2O_2 alone and in combination with sodium bicarbonate has been demonstrated in vitro for bacteria associated with gingivitis. In patients, however, delivery of these antibacterial agents necessitates use of mechanical instruments to gain access to deep pockets of anaerobic bacteria. No additional beneficial effects were observed with a subsequent oral rinse of saturated salts. In fact, use of saturated salt solutions increased gingival irritation and reduced patient compliance. Water has been successfully substituted for saturated salts, which should improve compliance.

In conclusion, adverse irritant effects from exposure to $\leq$ 3% H_2O_2 were rare. In animals, premalignant lesions and weak enhancement of tumor formation was reported with prolonged use of 30% H_2O_2. Use of solutions of $\leq$ 3% H_2O_2 in the oral cavity, even for prolonged periods of time should prove safe and beneficial in reducing plaque and supragingival microflora. For periodontal disease, therapeutic delivery of H_2O_2 requires mechanical access to subgingival pockets.

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