CLINICAL REPORT

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Safety issues relating to the use of hydrogen peroxide in dentistry

Laurence J. Walsh*

Abstract

Hydrogen peroxide is used widely in professionally and self-administered products. Hydrogen peroxide is a highly reactive substance which can damage oral soft tissues and hard tissues when present in high concentrations and with exposures of prolonged duration. This report provides an overview of health issues relating to the use of hydrogen peroxide, with an emphasis on safety with prolonged exposure to low concentrations of peroxide products. There is good evidence for the safety of hydrogen peroxide when used at low concentrations on a daily basis over extended periods of time, in self-administered oral health care products such as dentifrices and mouthrinses. These low concentrations neither damage oral hard or soft tissues, nor do they pose a significant risk of adverse long-term effects. Caution should be exercised with the increasingly higher concentration peroxide products used for 'walking' or 'power' bleaching due to the possibility of chemical irritation of oral soft tissues with injudicious use. The volumes of material and application times should be controlled carefully. Thorough education of patients is particularly important with self-applied gels because of the lack of professional supervision with such products. Such education is part of the duty of care of the dentist who supplies bleaching gels for at-home use.

Key Words: Dentifrice, bleaching, peroxide, toxicity.

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Introduction

Hydrogen peroxide has been used in dentistry for more than 70 years to bleach teeth, and in recent years the regular application of hydrogen peroxide has become more widely used as part of dental hygiene, particularly in combination with sodium bicarbonate ('baking soda'). In addition to dentifrices which contain hydrogen peroxide, in recent years there has been an increased use of self-applied bleaching agents which either contain or generate hydrogen peroxide, by both the dental profession and the general public. The most common ingredient used is carbamide peroxide, which when present at a concentration of 10 per cent releases 3.5 per cent hydrogen peroxide.[1]

While hydrogen peroxide can be toxic in high concentrations and with exposures of prolonged duration, concentrated (30-35 per cent) hydrogen peroxide solutions have been used for in-office bleaching treatments with no serious adverse soft and hard tissue effects observed clinically, other than the relatively common but self-limiting post-treatment sensitivity.[2] Nevertheless, there have been occasions when accidental ingestion of hydrogen peroxide products in the home has led to hospitalization and adverse health outcomes, and this should be kept in mind when assessing the relative safety of hydrogen peroxide-containing products for home (unsupervised) use.[3]

Much of the recent dental literature on the safety of hydrogen peroxide has focused on dentist-prescribed home bleaching, in which the teeth and oral soft tissues can be in contact with peroxide-type agents for extended periods of time. This provides a very different situation from either in-office bleaching or peroxide-containing dentifrices, from both dose and time standpoints (Table 1). For home oral health care products, such as mouthrinses and dentifrices, which contain low concentrations of hydrogen peroxide (1 per cent or less), the daily exposure to hydrogen peroxide will be much lower than when using self-administered bleaching agents which either contain or produce high levels of hydrogen peroxide for an extended period of time.[4,5-7]

The purpose of the present report is to summarize the current status of knowledge regarding the safety and efficacy of hydrogen peroxide in dentistry, with particular emphasis on its inclusion in mouthrinses, dentifrices and other home oral health care products, where safety issues with chronic administration are foremost. The information reviewed includes the

*Professor, Department of Dentistry, The University of Queensland.

Table 1. Exposure to hydrogen peroxide

Situation	Concentration used %	Duration of exposure
In-office bleaching (tooth surface)	30-35	5 minutes
Bleaching of stained root-filled tooth	30-35	30 minutes
'Walking bleach'	3.5	8 hours
Mouthrinse	1-3	5 minutes
Dentifrice	0.75	5 minutes

relevant medical/dental literature, together with searches of current international electronic registries of Material Safety Data Sheets for detailed hazard information.

Chemical hazard information

Hydrogen peroxide is a clear, colourless liquid with no odour. The molecular formula for hydrogen peroxide is H_2O_2 and its molecular weight is 34.0128. Thus, the molar concentration of a solution of 30 per cent hydrogen peroxide is 880 mmol/L. Hydrogen peroxide is relatively unstable and decomposes slowly to release oxygen. This decomposition is accelerated by light and heat, a feature which is exploited in various 'power-bleaching' techniques. Hydrogen peroxide is completely soluble in water and gives an acidic solution, the pH of which varies according to the concentration.[8] The pH of a 1 per cent solution is 5.0-6.0.

Hydrogen peroxide is an extremely strong oxidizing agent and is incompatible with a range of materials, including strong acids, acetone, alcohols (including ethyl alcohol), ammonia compounds, brass, bronze, chromium, copper, gold, iron, lead, magnesium and manganese.

Chemical toxicity

Under current national guidelines from WorkSafe Australia,[9] hydrogen peroxide is designated as a hazardous substance when present at concentrations above 5 per cent.

Direct exposure of skin or eyes to 30 per cent hydrogen peroxide may cause severe irritation or burns, while ingestion may be irritating to the oesophagus and stomach, causing bleeding and sudden distension.[8,9] Extensive tissue damage (vesicle formation and ulceration) can occur following inadvertent exposure of oral soft tissues to such concentrated solutions of hydrogen peroxide.[10] In a study using rats, 30 per cent hydrogen peroxide applied to tongue mucosa at 15 minute intervals produced extensive oedema of the submucosal tissues and tongue musculature. This had regressed within one day, leaving a large area of ulceration, which in turn healed completely within one week.[11] Of interest, the enzyme catalase (which breaks down hydrogen peroxide) was fully able to prevent the tissue reaction when applied to the tongue before hydrogen peroxide.

Variable responses of soft tissues when exposed to weaker hydrogen peroxide solutions have been reported in the literature. Direct and prolonged (30 minute) contact with 3 per cent hydrogen peroxide may cause irritation of the skin, oral and ocular mucosae, respiratory and digestive tracts, with burning, erythema and oedema.[12] There are isolated reports of patients who have developed oral ulcerations after using 3 per cent hydrogen peroxide for 1-2 minutes, 3-5 times daily,[13] while at lower concentrations, changes are less marked or inconspicuous even with continuous exposure. Martin *et al.*[14] applied 1 per cent hydrogen peroxide continuously in droplets to dog gingiva, and noted oedema in the epithelial layers after 6 hours, vacuolization after 24 hours, and destruction and sloughing of the cornified layers after 48 hours, but not frank ulceration.

A review of isolated case reports of accidental ingestion of hydrogen peroxide indicates that exposure can be associated with serious consequences. In a retrospective assessment of poisoning episodes, hydrogen peroxide accounted for a very small proportion (0.34 per cent) of all exposures reported to a regional poison centre over a 36 month period.[15] The paediatric population (<18 years) accounted for 71 per cent of hydrogen peroxide exposures, and ingestion was the most common route of exposure (83 per cent). Nausea and vomiting were the most common symptoms secondary to ingestion. Ocular and dermal exposures to dilute solutions resulted in transient symptoms without permanent sequelae. Most exposures by all routes resulted in a benign outcome (no effect or minor effect), however there was a trend toward more severe outcomes in those who ingested a concentration greater than 10 per cent.

For these reasons it is appropriate that products containing moderate concentrations of hydrogen peroxide (>5 per cent) carry warnings relating to avoiding both ingestion and contact of hydrogen peroxide solutions with skin or mucosal surfaces. The hazard rating for contact with 3 per cent hydrogen peroxide is rated as slight, while for 30 per cent hydrogen peroxide the hazard is rated as extreme because of the corrosive nature of strong hydrogen peroxide solutions.[16] For this reason, guidelines for tooth bleaching using concentrated solutions of hydrogen peroxide emphasize the need to prevent accidental exposure of gingival tissues to the solutions by use of a rubber dam. The risk of eliciting inflammation of the pulp because of percolation of hydrogen peroxide into the pulp via areas of exposed dentine or enamel fractures is also well recognized.[17,18]

Acute toxicity by ingestion

For accidental ingestion of hydrogen peroxide, the lethal dose (LD50) for oral ingestion of a 3 per cent solution of hydrogen peroxide is 90ml/kg in rats. However, studies of the effects of acute ingestion of hydrogen peroxide-containing products (other than solutions) are few. In a typical study, a single bolus of a commercial tooth whitener (35 per cent peroxide, 5g of tooth whitener/kg fasting body weight) was administered to rats by gavage. After two hours, respiration was suppressed and body temperature lowered and other signs of distress were noted. Three of 22 animals died within 48 hours as a result of gastric haemorrhage. Animals which received a bolus dose containing 10 or 15 per cent peroxide exhibited similar but milder symptoms.[19]

Acute toxicity by inhalation

Inhalation of hydrogen peroxide vapours from 3 per cent solutions may cause moderate respiratory tract irritation (chemical pneumonitis) and pulmonary oedema, while inhalation of vapours from 30 per cent solutions may cause severe irritation of the respiratory system. For this reason, safety information for hydrogen peroxide typically includes warnings to keep vapour and mist levels as low as possible. The WorkSafe Australia Occupational Exposure Limit (OEL) for vapours from solutions of hydrogen peroxide with a concentration greater than 3 per cent is 1ppm (1.5mg/m^3).[8,9] Importantly, mist and vapour are not significant problems when hydrogen peroxide is contained within a gel or solid formulation, such as a dentifrice.

The chemical reactions of hydrogen peroxide

Hydrogen peroxide is a member of a family of related molecules termed reactive oxygen species. This family includes a number of radicals (that is, species which contain one or more unpaired electrons), such as the superoxide (O_2.-), hydroxyl (HO.), peroxyl (ROO.) and alkoxyl (RO.) radicals. Hydrogen peroxide is formed by the reaction of superoxide (O_2.-) with itself, that is, a dismutation in which one molecule O_2.- is oxidized by the other. Within cells, hydrogen peroxide and the superoxide and hydroxyl radicals can be formed enzymatically and non-enzymatically.

Hydrogen peroxide can damage cells via several mechanisms and delay cell division. In the presence of chloride ions, the action of peroxidase on hydrogen peroxide produces hypochlorous acid (HOCl), which acts at low molar concentrations (10-20μmol/l) to damage proteins on cell membranes and destroy their function.[20] In addition, hydrogen peroxide can diffuse through lipid membranes and once inside the cell is able to react with iron, copper and other metallic ions to generate the highly reactive hydroxyl radical (HO.) and other oxidants.[21] These substances initiate chain reactions of lipid peroxidation which cause decomposition of the phospholipids of cellular membranes, which results in damage to lysosomal membranes and leakage of their destructive contents. The hydroxyl radical also damages the inner mitochondrial membrane, which can lead to the loss of viability of the cell. Superoxide can contribute to this process by reducing a cellular source of ferric to ferrous iron, and it is the latter which reacts with hydrogen peroxide to produce the more potent oxidizing agents.

As will be discussed below, DNA can be damaged by hydrogen peroxide and other reactive oxygen species. The formation of DNA strand breaks leads to activation of poly-ADP-ribose polymerase which in turn causes depletion of NAD and ATP, followed by calcium ion influx and eventually by cell lysis.[22]

In cell culture systems, in which protective enzyme systems (catalase and superoxide dismutase) are diminished or absent, hydrogen peroxide can exert marked cytotoxic effects. With a 30 minute exposure to a bolus dose of hydrogen peroxide in phosphate buffered saline at 37°C, cells can survive an exposure to 250μmol/l hydrogen peroxide, whereas at 350 and 500μmol/l exposure is lethal to a small fraction of cells. The oxidative stress causes decomposition of membranes with time- and dose-dependent leakage of damaging lysosomal hydrolytic enzymes. There is also cellular damage in the form of surface blebbing and increased autophagocytosis, which become more marked with higher doses of hydrogen peroxide. Of note, all these alterations are reversible, provided the cells are exposed to non-lethal doses.[23]

Gingival fibroblasts in cell culture are sensitive to toxic effects of hydrogen peroxide. Exposure to 100μmol/l of hydrogen peroxide (equivalent to 0.03 per cent) has been shown to cause an 80 per cent reduction in proliferation, as assessed by [^{3}H]-thymidine incorporation.[24] The relative sensitivity of pulpal, gingival and periodontal ligament cells to toxic effects of hydrogen peroxide is important given the potential for diffusion of hydrogen peroxide through dentine. The 50 per cent inhibitory dose (ID50) of hydrogen peroxide for succinyl dehydrogenase activity in cultured cells (580μmol/l) can be exceeded within one hour following application of 30 per cent hydrogen peroxide to dentine.[25]

In summary, hydrogen peroxide compromises several cellular functions, the end result of which can be cell death. While hydrogen peroxide may be injurious to tissue directly, secondarily derived oxidants such as hydroxyl radical as well as hypochlorous acid can also contribute to tissue injury.

Humans are well endowed with defences against hydrogen peroxide and other reactive oxygen species. These antioxidants, or free radical scavengers, include ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), beta-carotene, coenzyme Q10, and trace elements including selenium and zinc.[26] While these substances are effective at reducing the potential for cellular damage, the major antioxidant defensive mechanisms are the enzymes superoxide dismutase (SOD), glutathione peroxidase and catalase. SOD dismutases superoxide radicals to hydrogen peroxide, while catalase and glutathione peroxidase detoxify hydrogen peroxide. As would be expected from this, reactions elicited by hydrogen peroxide in a given tissue are influenced by the presence of SOD and catalase and the local concentration of metals such as iron and selenium.[26] Normal oral soft tissues, including dental pulp, contain SOD, and the level of activity is typically increased in inflamed tissues.[27]

Catalase is highly effective at preventing cellular damage from hydrogen peroxide. Catalase applied following the intracoronal application of concentrated hydrogen peroxide during bleaching procedures has been shown to eliminate residual hydrogen peroxide within three minutes.[28] Normal human serum contains catalase, and this provides a measure of protection from the adverse effects of hydrogen peroxide.[29] In contrast, the catalase activity of normal dental pulp appears to be low relative to other soft tissues.[30]

The mutagenic potential of hydrogen peroxide

Some concern has been expressed regarding both possible effects of hydrogen peroxide on cellular DNA and potentiating effects of hydrogen peroxide in the presence of known carcinogens.[31] The carcinogenicity of hydrogen peroxide has been rated differently by occupational health bodies in the United States, with the most recent rating being one of 'suspect carcinogen' by the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA).

According to the Council on Dental Therapeutics of the American Dental Association, concerns that hydrogen peroxide may enhance the effect of some carcinogens should be addressed by a combination of assay systems. According to their 1994 guidelines for assessing peroxide-containing oral hygiene products, 'at least one *in vitro* (either Ames Salmonella/microsome test or mammalian cell assay system) and one *in vivo* animal system (either the sister chromatid exchange assay or micronuclear test in bone marrow) should be included to estimate the genotoxic potential. If affirmative results are obtained from both *in vitro* and *in vivo* systems, then chronic animal testing for carcinogenicity using the hamster cheek pouch model is recommended.'

The following summarizes the available published information on the teratogenicity, reproductive effects and mutagenicity of hydrogen peroxide.

Cell and bacterial culture studies

At the level of the individual cell, hydrogen peroxide can permeate cells rapidly and inhibit adenosine triphosphate (ATP) synthesis via both glycolytic and oxidative phosphorylation (mitochondrial) pathways. In the glycolytic pathway, damage is limited to the step involving glyceraldehyde-3-PO_4 dehydrogenase (GAPDH). This results from both a direct attack on GAPDH and, indirectly, by a reduction in concentration of the GAPDH cofactor, nicotinamide adenine dinucleotide (NAD). This latter effect results from the activation of the enzyme, poly(adenosine diphosphate) (ADP)-ribose polymerase, an enzyme involved in deoxyribonucleic acid (DNA) repair.[20]

In cell culture studies, DNA damage in target cells has been reported at low concentrations of hydrogen peroxide (20-80μmol/l) in several cell types. Strand breaks and base hydroxylation have been observed, resulting from the generation of hydroxyl radicals from hydrogen peroxide, in the presence of metallic ions. DNA damage can result in either cell injury and death or mutations in the base sequence of DNA. The latter effects can lead to malignant transformations in cells cultured in the laboratory, and *in vivo* in T-lymphocyte deficient athymic mice.[20]

The potential mutagenic effects of hydrogen peroxide have been examined in laboratory studies. In one study, a vector plasmid carrying an *Escherichia coli* gene was used as a target for mutations. The plasmid was treated with a combination of hydrogen peroxide and iron/EDTA complex and propagated in *E. coli* bacterial host cells. The mutation frequency increased by up to 30-fold over spontaneous background levels with increasing concentrations of hydrogen peroxide.[32] These effects can be attributed to the potential for hydrogen peroxide, in the presence of metal ions, to cause breaks in DNA strands, resulting in gene loss or mutation. Strand breakage exposes DNA due to reduced protection from histones. 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, unless the affected DNA is repaired rapidly and accurately.

Of note, chromosome damage caused by hydrogen peroxide and iron in combination is enhanced by histidine, which complexes with iron. Thus, results from any laboratory tissue culture systems which use a medium containing histidine should be interpreted with caution.[5]

Finally, while hydrogen peroxide itself has been shown to be genotoxic in an oxidant-sensitive tester

strain of the bacterium *Salmonella typhimurium* (strain TA102), extensive testing has revealed that a hydrogen peroxide-generating tooth bleaching agent is neither genotoxic in this strain nor in mammalian cells. Furthermore, administration of 70mg/kg hydrogen peroxide per day for up to six months did not increase bone marrow sister chromatid exchange in Chinese hamsters.[5] Genotoxicity was also not seen in the TA102 strain with 267mmol/l hydrogen peroxide incorporated into a dentifrice. This may be explained in part by the presence of oxidant-trapping agents such as sorbitol in the dentifrice.

Animal studies

Weitzman *et al.*[33] have stated that hydrogen peroxide can induce pathologic changes frequently associated with neoplastic lesions (dyskeratosis) and that it may also augment carcinogenesis associated with 9,10-dimethyl 1,2 benzanthracene (DMBA). DMBA is a known carcinogen analogous to the polycyclic aromatic hydrocarbons which are found in tobacco. Their study used hamsters in which the buccal pouches were painted with 3 per cent or 30 per cent hydrogen peroxide solution twice weekly for 19 to 22 weeks, while DMBA was applied twice weekly on days other than when the hydrogen peroxide was applied. However, as pointed out subsequently by Marshall *et al.*,[5] the results of the Weitzman study are of marginal or no statistical significance because of the small number of animals utilized, and the weak effect observed even at the highest concentration of hydrogen peroxide. No differences were observed between control and experimental groups after 19 weeks. After 22 weeks, the incidence in the 3 per cent hydrogen peroxide treatment group tumour incidence was no different from that in the group treated with DMBA alone. While Weitzman *et al.*[33] claimed that hydrogen peroxide was a co-carcinogen in oral mucosa, both their own study and subsequent investigations have shown that at concentrations of 3 per cent or less, no co-carcinogenic activity or adverse effects are observed in the hamster cheek pouch model despite lengthy exposure to hydrogen peroxide.[5]

Of particular importance in the Weitzman study,[33] dentifrices formulated to deliver 0.75 per cent or 1.5 per cent hydrogen peroxide not only failed to show evidence of co-carcinogenicity after 20 weeks when administered concurrently with DMBA, but actually resulted in a significant delay in tumour development.

The only reports of complete carcinogenicity of hydrogen peroxide have come from the studies of Ito and coworkers.[34-36] In these investigations, gastric lesions were observed in mice after 10 weeks exposure to 0.4 per cent hydrogen peroxide in their drinking water. Of note, no lesions of the oral mucosa were reported in the animals. It is also noteworthy that a statistically significant positive result for tumour induction by hydrogen peroxide was only achieved by combining the data from males and females, which is contrary to the standard practice in these types of investigations. When analyzed separately by sex, as is the proper approach, there were no significant differences between control and hydrogen peroxide-treated groups.

In the studies by the Ito group,[34-36] the development of gastric tumours, described as hyperplastic lesions, varied among strains of mice exposed to hydrogen peroxide, and the extent of tumour induction was negatively correlated with catalase activity, which also differed among strains of mice. As noted earlier, catalase is an important natural defence mechanism against damage by hydrogen peroxide. Similar results (that is, complete carcinogenicity) have not been reported by other laboratories or in other animal models. The lesions observed in the Ito studies were mainly hyperplasia, which can revert to normal following removal of the stimulus.

Of particular interest in the Ito studies was the finding that lesions were more severe in the duodenum than in the gastric mucosa of C57BL/6 mice. The duodenum reportedly has the lowest organ catalase activity in these mice, and the spontaneous incidence of duodenal plaques has been reported to be as high as 70 per cent in normal (untreated) female mice.[37,38] In the Ito studies, the reversibility of the lesions induced in mice by hydrogen peroxide was demonstrated by replacement of drinking water containing 0.4 per cent hydrogen peroxide with distilled water for 10-30 days. Thus, the complete carcinogenicity of hydrogen peroxide was not demonstrated using standard assay and biochemical techniques. Moreover, the results obtained in a hypocatalasemic strain of mice (C57BL/6) are not relevant to humans.

Similar conclusions can be drawn from studies of the tumour-promoting effects of hydrogen peroxide. In mouse skin, both 6 per cent and 30 per cent hydrogen peroxide showed only very weak stimulatory effects on the development of papillomas. Moreover, when combined with the potent tumour-promoting agent DMBA for 58 weeks, 3 per cent hydrogen peroxide was inactive as a tumour promoter on mouse skin.[39] Even in studies of hamster cheek pouches, the lesions which did occur were not malignant tumours. After 14 months, only 12 per cent of animals showed changes and these lesions were papillary hyperplasias which can regress spontaneously.[40]

In accordance with these findings, there are published data regarding the effects of 10 per cent carbamide peroxide on genetic mutation after oral administration to mice. In these studies, genotoxicity, as measured by the well established mouse micronucleus test for mutagenicity, was negative.[41] In

summary, convincing evidence of tumour initiating, promoting or co-carcinogenetic activities of hydrogen peroxide in intact animals has not been presented.

Endogenous sources of hydrogen peroxide

Micro-organisms

Hydrogen peroxide can be produced by anaerobic micro-organisms in subgingival plaque. This can result in destructive periodontitis if the patient lacks catalase and the hydrogen peroxide produced cannot be degraded. Clinical studies of hypocatalasemic individuals have reported gingival necrosis and severe alveolar bone destruction. The gingival lesions in these patients were attributed to damage to the periodontal tissues from hydrogen peroxide generated by plaque organisms.[42] Aerobic organisms in these patients are protected from oxygen toxicity by their own production of SOD.

In addition to periodontopathic organisms, hydrogen peroxide can also be produced by certain anaerobic micro-organisms implicated in endodontic infections, such as *Streptococcus faecium*, while other streptococci, such as *Streptococcus faecalis*, are negative.[43] The cariogenic micro-organism *Streptococcus mutans* is negative for hydrogen peroxide production, although certain other common oral streptococci, namely *Streptococcus mitior* (*mitis biotype I*) and *Streptococcus sanguis I* and *II* (*sanguis* and *oralis*), are positive. Levels of hydrogen peroxide between 2.2 and 9.8mmol/l can be produced when these micro-organisms are grown aerobically, but only between 1.1 and 3.9mmol/l when grown anaerobically. These levels may be sufficient to inhibit the growth of other plaque bacteria. Of note, none of these peroxide-producing organisms are able to utilize hydrogen peroxide metabolically.[44]

Surprisingly, despite the fact that some oral streptococci produce hydrogen peroxide, free peroxide has not been found in dental plaque or salivary sediment despite streptococci being major components of their mixed bacterial populations. The absence of peroxide in plaque and sediment can be explained by dominance of destruction over formation by bacterial constituents. *Neisseria sicca*, *Haemophilus segnis, Haemophilus parainfluenzae, Actinomyces viscosus* and *Staphylococcus epidermidis* can destroy hydrogen peroxide readily, as can the mixed bacteria in salivary sediment and dental plaque, both of which contain relatively high numbers of these peroxide utilizing micro-organisms. The ability of the bacteria in plaque and sediment to degrade hydrogen peroxide is considerable. Peroxide removal is complete within 15 minutes even when the initial level is as high as 300mmol/l (equivalent to 10 per cent). Overall, there is a dominance of microbial peroxide removers over hydrogen peroxide producers in dental plaque,[44] although the balance is complicated by interactions between hydrogen peroxide, salivary lactoperoxidase and the thiocyanate ions.[45]

Human saliva

Molecular oxygen is produced when hydrogen peroxide comes into contact with human saliva. The oxygen is in a singlet state and is released via oxidation of hydrogen peroxide, a reaction which is catalysed by hypothiocyanate (OSCN-) bound to salivary peroxidase.[46] Supplementation of human saliva *in vivo* with low amounts (less than 1.0mmol/l) of hydrogen peroxide increases the concentration of salivary OSCN-. Elevated concentrations of OSCN- are strongly antimicrobial and may therefore be protective against dental caries and gingivitis. The elevated concentrations of OSCN- which produce inhibition of bacterial metabolism do not damage human cells.

Leukocytes and other cells

Hydrogen peroxide and its related radicals and lipid peroxides have been implicated in a range of human diseases. Evidence exists for a role for oxidant damage to tissues in the pathology of several human diseases, including rheumatoid arthritis, organ reperfusion injury, immune injury to the lung and kidney, and cerebral trauma or ischaemia.[47]

Neutrophils and macrophages can produce hydroxyl and superoxide radicals as well as hydrogen peroxide and hypochlorous acid as part of the normal defence mechanism against invading micro-organisms. These substances are destructive to most biological molecules, and are responsible for much of the 'bystander' damage inflicted by phagocytes on both micro-organisms and surrounding tissues at sites of infection or inflammation. As discussed above for bacteria, the extent of tissue damage in inflammation reflects the balance between free radicals generated and the antioxidant/radical scavenger protective defence systems. Hydrogen peroxide, in concentrations achieved in the proximity of stimulated phagocytes, can cause injury and lysis of cells. While hydrogen peroxide is not a normal constituent of blood or plasma, inflammatory reactions typically result in the activation and recruitment of phagocytic cells, such as neutrophils and macrophages, producing hydrogen peroxide and other mediators which can cause tissue injury.

Hydrogen peroxide effects on dental hard tissues

Enamel

Direct application of 3-30 per cent hydrogen peroxide to dental enamel has been used as part of the clinical management of non-carious enamel defects. Lightly discoloured or stained defects of the

enamel can be treated by bleaching the affected area without adverse effects on the dental pulp.[48]

Hydrogen peroxide has the potential to affect dental enamel because of the acidic pH of the solutions. The pH of concentrated solutions of hydrogen peroxide is stated by the various manufacturers as pH 5.0-6.0. This has been confirmed in independent studies in the literature.[49] Nevertheless, a study of teeth immersed in 6 per cent hydrogen peroxide failed to detect harmful effects on the tooth integrity as indicated by enamel solubility, hardness and tooth weight.[50]

Concentrated (30 per cent) solutions of hydrogen peroxide can reduce the microhardness of enamel and dentine. This reduction can be noted with exposure times as short as 5 minutes for the dentine and 15 minutes for enamel.[51] With more extended time periods, exposure of teeth in the laboratory to 30 per cent hydrogen peroxide for one week at 37°C has been shown to reduce the calcium/phosphorus (Ca/P) ratio in enamel, dentine and cementum,[52] indicating demineralization has occurred.

Several studies have shown that extended periods (5-30 minutes) of exposure of enamel to 35 per cent hydrogen peroxide causes a significant reduction in the adhesive bond strength of composite resin to enamel, which is more pronounced with prolonged exposure times. Scanning electron microscopic examination of fractured peroxide-treated specimens has revealed that the bond failures occur primarily at the bonding resin-enamel interface and are associated with areas of resin non-attachment and altered resin quality. It has been suggested that these changes are caused by the presence of residual peroxide or peroxide-related substances at or near the enamel surface.[53]

These findings have been confirmed in subsequent studies which examined the effects of 35 per cent hydrogen peroxide on enamel bonding of composite resins. Lower shear bond strengths were found and the effect was related to surface changes, in that removal of surface enamel restored bond strengths to normal levels.[54,55] The decrease in the adhesive bond strength of resin to 35 per cent hydrogen peroxide-treated enamel is not due to a change in the elemental composition of treated enamel surfaces, since studies have failed to find any major changes in the elemental composition of enamel exposed to 35 per cent hydrogen peroxide, other than an increase in nitrogen content.[56]

Direct effects of hydrogen peroxide on the morphology of human enamel have been documented. In a six week clinical study with the 'walking bleaching technique', hydrogen peroxide produced superficial destruction, with the appearance of patterning similar to the acid etching and the presence of some crystalline areas emerging from the body of the prisms.[57] As mentioned earlier, this may reflect a degree of etching resulting from the acidic pH of hydrogen peroxide solutions, although this has been disputed, with evidence both supporting this view[58] and challenging it.[59] Other studies have described varying degrees of surface porosity and alteration in enamel following exposure to high concentrations of hydrogen peroxide.[60]

An additional factor which should be recognized is the possibility that, following application, hydrogen peroxide can leach from the enamel surface and then exert an influence on saliva, dental plaque or restorative materials. This possibility has been examined directly in a study in which bovine enamel slabs were individually immersed in 2ml of 35 per cent hydrogen peroxide for 1, 3, 5, 30, or 60 minutes. After etching with 37 per cent phosphoric acid, leaching was carried out for intervals between 1 to 20 minutes, and up to 7 days. It was concluded that leaching of peroxide from enamel occurs rapidly and is enhanced by acid etching.[61]

Dentine

The effect of hydrogen peroxide on the inorganic composition of human dentine and cementum may be significant if concentrated solutions are used. In one study, powdered dentine and cementum were exposed to 3 per cent or 30 per cent hydrogen peroxide for 0.25, 1, 24, and 72 hours, and the degree of dissolution and the percentage of inorganic material for both dentine and cementum measured. Solutions of 30 per cent hydrogen peroxide significantly increased the solubility of both dentine and cementum, in a time-dependent relationship. The percentage of inorganic material remaining in the undissolved dentine and cementum increased with time. It was noted that 30 per cent hydrogen peroxide alters the chemical structure of the dentine and cementum, making them more susceptible to degradation via loss of organic components.[62] A later study demonstrated that 35 per cent hydrogen peroxide depletes dentine, with a weight loss in the order of 7 per cent dry weight over 24 hours, while a further investigation noted significant volume loss in both dentine and cementum after 4 or 8 weeks in an *in vitro* simulation of bleaching with hydrogen peroxide.[63]

Smear layers

Effects of hydrogen peroxide on dentine smear layers are critical to the potential of hydrogen peroxide to exert effects on dental pulp when in contact with exposed dentine. In one study, hydrogen peroxide was unable to remove the smear layer on dentine created by cavity preparation with a diamond bur, and the orifices of the dental tubules remained occluded by the smear layer.[64] This finding was

confirmed in a subsequent investigation in which the effectiveness of various agents in cleaning cut dentine surfaces was investigated. Attempts to remove the smeared layer of dentine after the preparation of a cavity using 3 per cent hydrogen peroxide were unsuccessful.[65]

However, in the situation where the smear layer has been removed by etching, application of 30 per cent hydrogen peroxide to dentine (via the root canal space) for extended periods (1-3 weeks) can increase the permeability of the teeth to a standard tracer organism (*S. faecalis*). Enhanced bacterial penetration through dentinal tubules by high concentrations of hydrogen peroxide may contribute to post-bleaching pathology, such as cervical resorption.[66]

Bonding of glass ionomer restorations

At high concentrations, hydrogen peroxide treatment of dentine can adversely affect the bonding of glass ionomer restorations. In one study, cylinders of Fuji Type II glass ionomer restorative cement were bonded to the superficial dentine layer of bovine incisor teeth that had previously been treated with 35 per cent hydrogen peroxide for 60 minutes. There was a highly significant reduction in bond strength of the cement when dentine was exposed to hydrogen peroxide as compared with saline. Scanning electron microscopic examination of fractured test specimens indicated that bond failure was cohesive in nature, suggesting that the hydrogen peroxide treatment adversely affected the setting process of the glass ionomer cement.[67]

A later study by the same group of investigators utilized the same experimental design but with composite resin in place of glass ionomer. In addition, specimens were stored in distilled water at 37°C for 7 days prior to shear bond strength testing. There was a significant reduction in shear bond strength with 35 per cent hydrogen peroxide treatment. Interestingly, water storage of hydrogen peroxide-treated specimens for one day prior to resin application appeared to restore the adhesiveness but not completely. Scanning electron microscopic examination revealed that the reduction in bond strength was related to alterations in the ability of the resin to attach itself to the enamel and to direct effects of hydrogen peroxide on the resin itself.[68] The latter can arise via liberation of hydrogen peroxide at the enamel-resin interface as a result of leaching, or by liberation of hydrogen peroxide from the composite resin material.[69]

Percolation into tooth structure

As mentioned above, hydrogen peroxide-induced cell injury has been implicated in the pathogenesis of external root resorption after bleaching of endodontically treated teeth. This bleaching is typically performed with 30 per cent hydrogen peroxide solutions. A study which provided insight into this phenomenon used an *in vitro* model to quantify the degree of hydrogen peroxide penetration during bleaching procedures. Artificial defects of the cementum covering the cemento-enamel junction were created in 22 extracted human premolars. After 15 minutes, hydrogen peroxide could be detected in the medium surrounding all the tested teeth. It was noted that cervical root permeability to hydrogen peroxide could be as much as 82 per cent of the total amount applied.[70]

These findings were extended in a follow-up study in which extracted human premolars were treated endodontically and bleached intracoronally with a concentrated (30 per cent) hydrogen peroxide solution. The teeth were divided into three groups: one group with no cementum defects at the cemento-enamel junction, one group with artificial cementum defects at the cemento-enamel junction, and another group with artificial cementum defects at the middle third of the root. The radicular penetration of 30 per cent hydrogen peroxide in the three groups was assessed directly and compared using an *in vitro* model. Radicular penetration of hydrogen peroxide was found in all of the groups tested. Of note, the penetration of hydrogen peroxide was significantly higher in teeth with cementum defects at the cemento-enamel junction than in those without defects.[71] It is now realized that all teeth with cementum defects can be expected to show rapid penetration of hydrogen peroxide.[72]

Dental pulp

As noted previously, hydrogen peroxide in high concentrations can exert highly irritant effects on soft tissues, and this includes the dental pulp. Application of 30 per cent hydrogen peroxide to dental pulp of rat incisor teeth has been shown to produce inflammation associated with a slowing of the circulation and partly irreversible capillary stasis.[73] In a similar study, which examined histologically the effect of direct application of 30 per cent hydrogen peroxide to the pulp tissue of dog teeth, the irritant effects included obliteration of odontoblasts, haemorrhage, resorption and inflammatory infiltration. Some of these pulpal changes were reversible after extended periods (60 days).[74] Other studies have determined that 10 per cent and 30 per cent hydrogen peroxide solutions can penetrate to the dental pulp within 15 minutes of application to the enamel surface.[75] A further consideration is that 30 per cent hydrogen peroxide treatment of dentine may elicit a pulpal response by altering the extent of microleakage around a composite resin restoration.[76]

Composite resins

Hydrogen peroxide in high concentrations for extended periods can influence the mechanical properties of composite resin restorative materials. In one study, representative microfill, hybrid, and posterior composite resins were immersed in 30 per cent hydrogen peroxide for one hour or one week at 37°C. An equal number of specimens stored in de-ionized water served as controls. Qualitative examination of bleached specimens revealed a marked change in colour, especially the microfilled composite resins. There was no significant difference in tensile strength between controls and exposed samples at either time, except for a diminished strength of microfilled composite resins stored in 30 per cent hydrogen peroxide for one week.[77]

Zinc oxide-eugenol materials

Prolonged exposure to 10 per cent hydrogen peroxide has been shown to adversely affect the surface morphology of reinforced zinc oxide-eugenol (IRM) restorations. When samples of IRM were placed in hydrogen peroxide for 1, 3 or 7 days at 37°C, numerous cracks were found and the samples appeared swollen when compared with controls maintained in phosphate buffer. There were no changes in levels of zinc oxide compared with controls.[78]

Clinical studies of hydrogen peroxide in dentistry

Mouthrinses

There have been few studies of high concentration hydrogen peroxide mouthrinses used for treatment of oral soft tissues. In one such study, 122 patients with post-extraction pain were treated with a 6 per cent hydrogen peroxide mouthrinse, which was applied with a syringe and needle into the extraction site in consecutive daily sessions.[79] Pain was relieved after one to eight rinsings (average 2.64), with no reports of soft tissue irritation. In a separate study, mucosal status was evaluated in 35 normal subjects who rinsed vigorously with 1.5 per cent hydrogen peroxide or saline four times daily for two weeks. In the saline group, no significant changes were noted, while in the hydrogen peroxide group, erythema and mucosal irritations were observed occasionally.[80]

In a further study conducted over an 18 month period, 25 subjects undergoing orthodontic treatment with fixed appliances used a mouthrinse containing 1.5 per cent hydrogen peroxide once daily in addition to toothbrushing. Particular attention was paid to the occurrence of mucosal irritations or staining of the teeth or tongue. Assessments were made before appliances were placed (baseline) and 1, 3, 6, 9, 12 and 18 months after appliances were placed. The peroxide group (25 subjects) had significantly fewer sites with plaque or gingivitis than the control group (34 subjects) throughout the 18 month period. Of note, no generalized mucosal irritations or clinically significant staining of the tongue or teeth were noted in either group during the study, indicating the safety of the hydrogen peroxide regimen used.[81] An identical result regarding the soft tissue safety of 1.5 per cent hydrogen peroxide used as a daily rinse over two years was found in a follow-up study by the same investigator.[82] Lack of adverse soft tissue effects was also a feature of studies which have examined the use of hydrogen peroxide as a subgingival irrigant.[83]

In a double-blind, three month clinical study which examined the intra-oral safety and efficacy of a hydrogen peroxide mouthrinse in reducing the extrinsic stain produced by a chlorhexidine mouthrinse, extrinsic tooth stain and plaque accumulation scores were determined in 119 healthy adult subjects at baseline and at 30-, 60- and 90-day intervals. While the mouthrinse significantly reduced the extrinsic tooth staining produced by chlorhexidine, no adverse soft tissue side-effects were observed or reported throughout the study.[84]

Further insights into soft tissue reactions can be gained from studies in which hydrogen peroxide mouthrinses have been used as a replacement for conventional oral hygiene measures in the post-surgical healing phase. In a typical study involving 15 patients with dental splints for jaw fractures and intermaxillary wiring, a 1 per cent hydrogen peroxide mouthrinse was used daily for five weeks, and the Plaque Index recorded at days 7, 21, and 35 post-operatively. While plaque reductions of up to 22 per cent were observed, no adverse soft tissue side effects were recorded.[85]

More recently, the side-effect profile of rinses which combine povidone-iodine and hydrogen peroxide has been evaluated. In these studies, there have been no problems with soft tissue irritation,[86-88] a finding which confirms the lack of soft tissue injury in clinical studies of low concentration (1 per cent) hydrogen peroxide solutions used either as mouthrinses or as subgingival irrigants.[89]

Dentifrices

Hydrogen peroxide-containing dentifrices have been shown to exert beneficial effects on subgingival flora in patients with periodontitis, without causing irritations or other adverse soft tissue changes.[83,90-92] Similar comments can be made regarding dentifrices which contain sodium bicarbonate (baking soda) as well as 3 per cent hydrogen peroxide.[93] Long-term studies of patient compliance with, and acceptance of, hydrogen peroxide-containing dentifrices have failed to show evidence of adverse effects. In a

typical study conducted over a two year period, 58 per cent of subjects used the dentifrice 4-7 days a week during the entire study, and 96 per cent felt that their periodontal status improved.[94]

In recent years, there have been several detailed studies of the safety of dentifrices containing hydrogen peroxide and sodium bicarbonate. In a study by Fischman and co-workers,[95,96] laboratory, clinical, and microbiological analyses examined the influence of daily use of a dentifrice delivering 0.75 per cent hydrogen peroxide and 5 per cent baking soda. Laboratory studies using calcium radiolabelled teeth indicated that the dentifrice did not decalcify enamel or bleach teeth. Over the course of a six month period, 62 subjects using the hydrogen peroxide/baking soda dentifrice and 21 subjects using a control dentifrice were examined for oral soft tissue change and hard tissue alterations. No soft tissue changes attributable to the use of either dentifrice were noted. A similar lack of adverse effects and excellent patient acceptance has been documented in other studies of peroxide-containing dentifrices,[97,98] chewing gums,[99] and mouth-rinses.[81,94-96,100,101] In short, prolonged use of hydrogen peroxide at concentrations of 1.5-3 per cent in oral hygiene products has not been shown to cause adverse soft tissue changes despite having beneficial effects on plaque, gingivitis and wound healing.

Bleaching products

Products with high concentrations of hydrogen peroxide are employed for a variety of tooth bleaching procedures in custom trays as part of the so-called 'walking-bleach' technique, while concentrated solutions of hydrogen peroxide have been used for in-office procedures such as 'power bleaching', where the decomposition of the solution (and thus oxygen release) is accelerated by the application of heat, coherent (laser) light or conventional (non-coherent) light. With such high concentration products, the likelihood of irritant reactions is greatly increased. Risk areas include both the oral mucosa and extra-oral sites (such as the eye), since the latter may become involved through residues left on the hands which may be transferred accidentally to the eyes. Accidental exposure of the eyes to concentrated (30 per cent) hydrogen peroxide is likely to result in chemically-induced ulceration and irritation,[8,9] and this emphasizes the importance of both protective eye-wear in the dental surgery, and careful handcare and hygiene for patients who load their own special applicator trays without supervision from the dentist.

Tooth bleaching requires intimate contact with tooth structure, and while custom trays are employed, gingival irritation may result when the bleaching material escapes the holding tray and remains in the cervical area, where salivary clearance is typically poor. The resulting chemical irritation results in post-treatment sensitivity in the treated area. The gingival soft tissue response may be worsened by dehydration, which occurs as a result of prolonged exposure to the anhydrous glycerin base used in most bleaching gels.[5]

Soft tissue irritation from peroxide products is much more likely to occur when the epithelium overlying the gingival (and other oral) soft tissues is abnormally thin or permeable. This may occur in a variety of conditions where there is frank inflammation of the tissues (such as when gingival lesions of oral lichen planus or vesiculobullous diseases are present), or when the epithelium is atrophic (such as following anticancer chemotherapy or a consequence of prolonged xerostomia). In such patients, it would be prudent to avoid exposure to chemical irritants of all types, and thus moderate to high concentration peroxide products for tooth bleaching or mouth-rinsing should not be used. In contrast, few problems tend to occur with dentifrices because of the much lower concentrations involved (Table 1).[96] In the author's oncology practice, hydrogen peroxide mouthrinses are not used because of the tendency for soft tissue irritation to occur in patients with delicate (and often frankly ulcerated) oral mucosa, and this is consistent with hospital nursing practices in general.[80]

Conclusions

Possible concerns with the intra-oral use of hydrogen peroxide are effects on soft tissues, teeth and dental restorative materials. In high concentrations, hydrogen peroxide may have transient effects on the tooth itself and may also affect some dental materials. Soft tissues exposed to high concentrations of hydrogen peroxide for short periods may show chemical injury in the form of erythema or mucosal sloughing, while exposure for prolonged periods may cause inflammation or hyperplasia.

These changes have not been documented with dentifrices, which typically contain only a quarter of the concentration of hydrogen peroxide used commonly as a mouthrinse for debriding the oral cavity (3 per cent). In fact, studies in which hydrogen peroxide at concentrations of 3 per cent or less have been used daily for up to six years, have reported occasional and transitory irritant effects only in a small number of subjects with pre-existing ulceration, or when salt solutions were concurrently administered. This is in sharp contrast to the effects of bleaching agents which either use or generate high levels of hydrogen peroxide, which can produce localized oral toxicity following sustained exposure if mishandled.

This emphasizes the need for thorough education of patients before supplying bleaching gels for home use.

In contrast, there is strong evidence for the safety of low-concentration hydrogen peroxide-containing products when used on a daily basis over an extended period of time. From the available evidence, it can be concluded that the low concentrations of hydrogen peroxide present in dentifrices neither damage oral hard and soft tissues, nor do they pose a significant risk of adverse long-term effects, such as cancer.

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Address for correspondence/reprints:
Professor L. J. Walsh,
School of Dentistry,
The University of Queensland,
Turbot Street,
Brisbane, Queensland 4000.