

Literature review of the role of hydroxyl radicals in chemically-induced mutagenicity and carcinogenicity for the risk assessment of a disinfection system utilizing photolysis of hydrogen peroxide

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We have developed a new disinfection system for oral hygiene, proving that hydroxyl radicals generated by the photolysis of 1 M hydrogen peroxide could effectively kill oral pathogenic microorganisms. Prior to any clinical testing, the safety of the system especially in terms of the risk of carcinogenicity is examined by reviewing the literature. Previous studies have investigated indirectly the kinds of reactive oxygen species involved in some sort of chemically-induced mutagenicity *in vitro* by using reactive oxygen species scavengers, suggesting the possible involvement of hydroxyl radicals. Similarly, possible involvement of hydroxyl radicals in some sort of chemically-induced carcinogenicity has been proposed. Notably, it is suggested that the hydroxyl radical can play a role in heavy metal-induced carcinogenicity that requires chronic exposure to the carcinogen. In these cases, hydroxyl radicals produced by Fenton-like reactions may be involved in the carcinogenicity. Meanwhile, potential advantages have been reported on the use of the hydroxyl radical, being included in host immune defense by polymorphonuclear leukocytes, and medical applications such as for cancer treatment and antibiotics. From these, we conclude that there would seem to be little to no risk in using the hydroxyl radical as a disinfectant for short-term treatment of the oral cavity.

Key Words: hydroxyl radical, mutagenicity, carcinogenicity, photolysis, hydrogen peroxide

Applying the oxidative power of hydroxyl radicals to disinfection systems has been attempted by utilizing sonolysis of water and photolysis of hydrogen peroxide in our laboratory.⁽¹⁻³⁾ In the latter system, designed for oral hygiene, hydroxyl radicals generated by the photolysis of 1 M hydrogen peroxide increased with laser irradiation time and could kill oral pathogenic bacteria in as little as one to three minutes. In other words, the time-dose relationship of this system shows that it is very effective. Therefore, we concluded that a disinfection technique using artificially generated hydroxyl radicals could be applied to the treatment of various oral infectious diseases. Fig. 1 shows a schematic illustration of a device for the treatment of dental infectious diseases. Hydrogen peroxide is released from the tip of the device to the lesion site concomitantly with laser irradiation through an optical fiber. One molar hydrogen peroxide (H₂O₂) used in the system corresponds to approximately 3% which is a concentration used in oral cavity as a disinfectant. A subcommittee of US Food and Drug Administration (FDA, 2003) also concluded that hydrogen

peroxide is safe at concentrations of up to 3%. While the experimental data suggest a mutagenic effect of hydrogen peroxide, the Subcommittee's review indicates that, at concentrations of up to 3% in oral care products, the risk appears to be especially minimal and hydrogen peroxide is safe for its intended use (FR Doc 03-12783). Regarding a safety aspect of the system, we have conducted toxicity studies using rabbits and rats (unpublished data). In the rabbit study, the locally injurious properties of the hydroxyl radical generation system was evaluated by using a bone wound model. As a result, the single treatment with the disinfection system utilizing the photolysis of H₂O₂ exerted no significant effect on the healing of bone wound produced on the femur and the tibia. In the rat study, oral cavity was exposed to H₂O₂ with laser irradiation once a day for three days, resulting in that no abnormalities were visually found in oral mucous membrane. In addition, prior to clinical testing, the safety of the system utilizing hydroxyl radical generation must be evaluated in terms of the risk of carcinogenicity. However, it is very hard to assess mutagenicity of the system *in vitro* by bacterial reverse mutation assay and chromosome aberration test, because bacterial and mammalian cells should be continuously exposed to 1 M H₂O₂ with laser irradiation for certain period of time. Bibliographically, although there have been many studies on oxidative cellular damage such as membrane lipid peroxidation, protein denaturalization, and nucleic acid modification in relation to reactive oxygen species (ROS) including the hydroxyl radical,⁽⁴⁻⁶⁾ the relevant effects of the radical on carcinogenicity have not been clearly elucidated possibly because of its extremely short-half life, approximately 10⁻⁹ s.^(7,8) To assess the risk of carcinogenicity from using hydroxyl radicals to treat oral infections, this paper examines the possible involvement of the hydroxyl radical in chemically-induced mutagenicity and carcinogenicity by reviewing the literature.

Hydroxyl Radical and Mutagenicity

There have been many reports on the possible role of the hydroxyl radical in chemically induced mutagenicity/genotoxicity. de Kok *et al.*,⁽⁹⁾ used electron spin resonance (ESR) spectroscopy to study oxygen radicals generated by synthetic fecapentaene-12 (FP-12), which belongs to a class of potent fecal mutagens and has been suggested to play an initiating role in colon carcino-

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genesis. Although no specific conditions that resulted in the direct formation of oxygen radicals from FP-12 could be defined, the formation of hydroxyl radical spin adducts was demonstrated after peroxidation of FP-12 in incubations with the spin-trapping agent 2,2,6,6-tetramethyl-piperidine. Furthermore, in mutagenicity assays to examine the effect of removing hydroxyl radicals with respect to the genotoxic potential of FP-12, the scavenging reduced the number of revertants in *Salmonella* strains TA100, TA102 and TA104, suggesting a partial involvement of the hydroxyl radical in the mutagenic effect with the formation of superoxide anions after the peroxidation of FP-12, which is subsequently converted to hydroxyl radicals by the iron-catalyzed Haber-Weiss reaction. Chlopkiewicz *et al.*⁽¹⁰⁾ examined whether the hydroxyl radical contributes to the mutagenicity of 2-chloropyridine in *Salmonella typhimurium*. They found that the mutagenicity was effectively suppressed by scavengers of the hydroxyl radical and was not inhibited by the addition of superoxide dismutase (SOD) or catalase. However, the results did not clarify whether the mutagenicity of 2-chloropyridine was caused by hydroxyl radicals or whether other oxygen species generated intracellularly were involved, because SOD or catalase added to the medium could not scavenge the superoxide anion or H₂O₂ generated within the cell. In a study by Edenharder and Grunhage, the mutagenicity caused by *t*-butyl hydroperoxide or cumene hydroperoxide in *Salmonella typhimurium* TA102 was effectively reduced by some flavonols.⁽¹¹⁾ However, although they concluded that in the *Salmonella*/reversion assay with strain TA102, antimutagenic activities of flavonoids against the peroxide mutagens *t*-butyl hydroperoxide and cumene hydroperoxide are mainly caused by radical-scavenging effects, it was not clarified which radical species were involved. Similarly, hydrogen peroxide-induced mutagenicity in *Salmonella typhimurium* TA102 was assumed to be attributable to hydroxyl radicals.⁽¹²⁾ In the study, it was proposed that the hydrogen peroxide-induced mutations in the *Salmonella* cells are caused by hydroxyl radicals generated by iron ions closely associated with DNA, because protection against the mutagenicity was achieved with ascorbic acid and dimethyl sulfoxide (DMSO), both effective scavengers of hydroxyl radicals. Yoshida *et al.*⁽¹³⁾ reported the mutagenicity of *p*-aminophenol (*p*-AP) in *Escherichia coli* WP2uvrA/pKM101 and its relevance to oxidative DNA damage. Since the mutagenic activity of this compound was suppressed with the addition of DMSO or catalase, they suggested the involvement of active oxygen species in the mutagenic process induced by *p*-AP. Thus, they speculated hydroxyl radicals derived from the reaction between hydrogen peroxide and Fe(III) (Fenton reaction) to be acting in *Escherichia coli* WP2uvrA/pKM101 during the mutation by *p*-AP.

Beside the mutagenicity in bacteria, oxidative DNA damage and chromosomal aberrations have been reported in yeast and mammalian cells. Akman *et al.*⁽¹⁴⁾ reported DNA base modifications induced in isolated human chromatin by the NADH dehydrogenase-catalyzed reduction of doxorubicin, an antineoplastic benzanthroquinone. Since the reduction of doxorubicin caused hydroxyl radical production, it was suggested that the flavoenzyme-catalyzed redox cycling of doxorubicin generates typical hydroxyl radical-induced base modifications in the DNA of isolated human chromatin, which might be a possible mechanism for the mutagenicity of doxorubicin *in vivo*. Dizdaroglu *et al.*⁽¹⁵⁾ found that phorbol-12-acetate-13-myristate (PMA)-activated human polymorphonuclear leukocytes (PMNs) caused base modifications in target cell DNA *in vivo*, suggesting that potentially promutagenic bases contribute to the mutagenicity of activated PMNs via the generation of reactive oxygen species including the hydroxyl radical. With regard to chromosomal aberrations, it was reported that of five oxidative mutagens, methyl viologen (paraquat), mitomycin C, phenylhydrazine, cumene hydroperoxide and hydrogen peroxide, only hydrogen peroxide increased the frequency of both intrachromosomal recombination and interchromosomal recombi-

nation in the yeast *Saccharomyces cerevisiae*.⁽¹⁶⁾ A role for the hydroxyl radical in the effect of hydrogen peroxide on recombination was indicated by the ability of the radical scavenger DMSO to significantly inhibit the induction of both intrachromosomal and interchromosomal recombination by hydrogen peroxide.

Overview of the Hydroxyl Radical and Mutagenicity

As described above, there have been many reports on the involvement of hydroxyl radicals in mutagenicity in bacteria, yeast and mammalian cells. Table 1 summarizes these studies. Of these, only one tried to detect reactive oxygen species including the hydroxyl radical by using ESR spin trapping to elucidate the underlying mechanism of FP-12-induced mutagenicity in *Salmonella*.⁽⁹⁾ The rest of the studies examined indirectly the kinds of ROS involved in chemically-induced mutagenicity or DNA base modifications in bacteria, yeast or human cells by using ROS scavengers such as DMSO for the hydroxyl radical and catalase for hydrogen peroxide. These findings suggest the possible involvement of hydroxyl radical in chemically-induced mutagenicity *in vitro*.

Hydroxyl Radical and Carcinogenicity

The possible involvement of hydroxyl radicals in chemically induced carcinogenicity has been reported in several papers. The hydroxyl radicals produced by Fenton-like reactions may be involved in the carcinogenicity of iron (Fe), copper (Cu) and other heavy metals such as chromium (Cr) and arsenic. Fe and Cu are essential elements for the functions of various enzymes and cellular proteins, and are regulated by certain biomolecules such as transferrin receptor for Fe and Cu transporter-1 for Cu.^(17,18) It was reported that accumulation of Fe is associated with colorectal cancers in humans, and a genetic hemochromatosis is associated with increased risk of human hepatocellular tumors.⁽¹⁹⁾ Liver tumor promotion was also evident after Fe overloading in a rat two-stage hepatocarcinogenesis study.^(20,21) It was also proposed that lipid peroxidation initiated by Fe-mediated oxidative stress possibly including hydroxyl radicals is associated with renal carcinogenesis.^(22,23) With regard to Cu, it was reported that increased Cu storage due to impaired cellular Cu transporters can cause serious liver diseases.⁽²⁴⁾ Especially, liver cell injury caused by generating ROS including hydroxyl radicals through excess Cu accumulation can cause tumor promotion, possibly by inducing oxidative DNA damage.⁽²⁵⁻²⁷⁾

Ye *et al.*⁽²⁸⁾ reported that Cr(VI) in combination with glutathione reductase enhanced the activity of nuclear factor (NF)- κ B in Jurkat cells. This activation of NF- κ B was decreased by a metal chelator, diethylenetriaminepentaacetic acid or catalase, but increased by SOD. ESR measurements using 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) confirmed that the incubation of Cr(VI) with Jurkat cells in the presence of glutathione reductase generated hydroxyl radicals. In addition, the hydrogen peroxide enhanced hydroxyl radical generation and also enhanced Cr(V) formation, suggesting Cr(VI)-mediated NF- κ B activation to be involved in the mechanism of Cr(VI)-induced carcinogenicity. According to Liu *et al.*,⁽²⁹⁾ the Cr(VI)-glutathione complex is able to generate hydroxyl radicals in the presence of molecular oxygen in aqueous medium. Since a catalase inhibited the production of hydroxyl radicals while hydrogen peroxide enhanced it, the hydroxyl radical was generated via a Fenton-like reaction. The results imply the involvement of the Cr(IV)-mediated production of the hydroxyl radical in the mechanism of Cr(VI)-induced carcinogenesis. More recently, Hojo *et al.*⁽³¹⁾ successfully detected the hydroxyl radical in the blood of mice after acute Cr(VI) intake by applying ESR spin-trapping. The ESR spectrum of the blood of mice given 4.8 mmol Cr(VI)/kg body weight revealed 7.37 μ M DMPO-OH, a spin adduct of DMPO and the hydroxyl radical, supporting the

Table 1. A list of studies on possible involvement of ROS including the hydroxyl radical in mutagenicity

Author	Mutagen	Assay	Brief summary
de Kok 1992	Fecapentaene-12	Bacterial reverse mutation	Mutagenicity induced by fecapentaene-12 in <i>Salmonella</i> strains was suppressed by scavengers of hydroxyl radical.
Akman 1992	Doxorubicin	DNA base modification	DNA base modifications were induced in isolated human chromatin by NADH dehydrogenase-catalyzed reduction of doxorubicin, which causes hydroxyl radical production.
Dizdaroglu 1993	Phorbol-12-acetate-13-myristate (PMA)	DNA base modification	Exposure of target cells to PMA-activated human PMNs caused DNA base modifications in the cells.
Chlopkiewicz 1993	2-Chloropyridine	Bacterial reverse mutation	Mutagenicity induced by 2-chloropyridine in <i>Salmonella</i> strains was suppressed by scavengers of hydroxyl radical.
Brennan 1994	Hydrogen peroxide	Chromosomal aberration	Hydrogen peroxide-induced intrachromosomal and inter-chromosomal recombination in <i>Saccharomyces cerevisiae</i> were suppressed by DMSO.
Yoshida 1998	p-Aminophenol	Bacterial reverse mutation	Mutagenicity induced by p-aminophenol in <i>E. coli</i> WP2uvrA/pKM101 was suppressed by DMSO or catalase.
Edenharder and Grunhage 2003	t-Butyl hydroperoxide (BHP), Cumene hydroperoxide (CHP)	Bacterial reverse mutation	Mutagenicity induced by BHP or CHP in <i>Salmonella typhimurium</i> TA103 was reduced by some flavonols, which possess radical-scavenging effects.
Grey 2003	Hydrogen peroxide	Bacterial reverse mutation	Mutagenicity induced by hydrogen peroxide in <i>Salmonella typhimurium</i> TA102 was reduced by ascorbic acid and DMSO, both of which are effective scavengers of hydroxyl radical.

idea that the hydroxyl radical is a putative cause of Cr(VI)-induced carcinogenesis. Epidemiological studies have indicated that people exposed to high levels of arsenic are prone to skin, bladder, liver, and lung cancers,⁽³¹⁾ and the skin is one target for arsenic toxicity such as keratinocytic tumors including basal cell carcinoma and squamous cell carcinoma, since it was reported that organic and inorganic arsenic can be absorbed through the skin in animals and humans.⁽³²⁾ Regarding the underlying mechanism by which arsenic induces skin cancer, Shi *et al.*⁽³³⁾ examined the chemical nature of specific ROS, studied the interrelationship among these species, and identified the specific species responsible for the subsequent DNA damage in a spontaneously immortalized keratinocyte cell line. They detected the formation of superoxide anion and hydrogen peroxide in keratinocytes incubated with arsenite(III) concomitant with DNA damage. Catalase eliminated hydrogen peroxide and reduced the arsenite(III)-mediated DNA damage. In contrast, SOD significantly increased the arsenite(III)-mediated damage possibly by enhancing the production of hydrogen peroxide and hydroxyl radicals. Since sodium formate, a competitive scavenger for the hydroxyl radical, and deferoxamine, a metal chelator, both reduced the DNA damage, exposure to arsenite(III) may generate superoxide anions and hydrogen peroxide, and hydroxyl radicals derived from hydrogen peroxide, may play an important role in arsenic-induced skin carcinogenicity. However, unlike Cr and arsenic, the involvement of hydroxyl radicals in vanadium-induced carcinogenesis was reported to be minimal.⁽³⁴⁾ A correlation between exposure to airborne vanadium particles and the incidence of cancer in residents of metropolitan areas has been indicated by epidemiological studies.^(35,36) It was reported that mutations and DNA-protein crosslinks were induced by vanadate(V) in cultured mammalian cells,⁽³⁷⁾ and the transforming activity of vanadate(V) was found in BALB/3T3 cells,⁽³⁸⁾ suggesting that vanadate(V) may act both as an initiator and as a promoter of morphological transformation in cultured mammalian cells. More recently, Ding *et al.*⁽³⁴⁾ reported that the toxicity and carcinogenicity of vanadium arise from elevated levels of ROS leading to activation of the transcription factor activator protein-1 (AP-1). To investigate the possible activity of vanadium in the activation of AP-1, they treated mouse epidermal JB6P1 cells stably transfected with an AP-1 luciferase reporter plasmid with various concentrations of vanadate(V). SOD and catalase inhibited AP-1 activation induced by vanadate(V), indicating the involvement of superoxide anions and/or hydrogen peroxide in the mechanism of vanadate(V)-induced AP-1 activation. However,

sodium formate failed to inhibit vanadate(V)-induced AP-1 activation, suggesting that vanadium, like other classes of tumor promoters, transactivates AP-1-dependent gene expression which might be attributable to the generation of superoxide anions and hydrogen peroxide, but not hydroxyl radicals.

Beside the involvement of ROS in the carcinogenicity of heavy metals, Elliot *et al.*⁽³⁹⁾ reported that carcinogenicity observed in rodents with certain peroxisome proliferators might be linked with ROS including the hydroxyl radical. In their study, ESR using the spin trap DMPO was employed to measure hydroxyl radical production in liver peroxisome-enriched fractions isolated from male Alpk/Ap rats administered chemicals such as di(2-ethylhexyl)phthalate, clofibrate and methyl clofenapate, all of which are known to cause peroxisome proliferation. The results showed that the rate of production of hydroxyl radicals was greater in fractions from the treated animals, raising a hypothesis linking such ROS to carcinogenicity observed in rodents treated with certain peroxisome proliferators.

Overview of the Hydroxyl Radical and Carcinogenicity

Table 2 summarizes studies on the involvement of ROS including the hydroxyl radical in chemically-induced carcinogenicity. Only one study, where the possible involvement of ROS in vanadium-induced carcinogenicity was examined, expressed skepticism about the involvement of the hydroxyl radical.⁽³⁴⁾ All the other studies proposed the hydroxyl radical to be involved in chemically-induced carcinogenicity. Notably, the carcinogenicity of heavy metals such as Cr and arsenic may involve hydroxyl radicals produced by Fenton-like reactions.⁽²⁸⁻³⁰⁾

Conclusion

Despite a lack of direct evidence *in vitro*, the hydroxyl radical is likely a causative factor for bacterial reverse mutations and DNA base modifications in yeast and mammalian cells induced by mutagens, because the scavenging of hydroxyl radicals resulted in the cancellation or attenuation of these mutagenic responses. Similarly, the hydroxyl radical possibly plays a role in heavy metal-induced carcinogenicity since these metals can mediate Fenton-like reactions with hydrogen peroxide, resulting in hydroxyl radicals as illustrated in Fig. 2. The generation of superoxide anion following the exposure of cells to heavy metals such as Cr and arsenic gives rise to hydrogen peroxide via a dismutation reaction

Table 2. A list of studies on possible involvement of ROS including the hydroxyl radical in carcinogenicity

Author	Carcinogen	Brief summary
Elliot 1986	Peroxisome proliferator	The rate of production of hydroxyl radicals was greater in fractions from the male rats treated with a peroxisome proliferator such as di(2-ethylhexyl)phthalate, clofibrate or methyl clofenapate, raising a hypothesis linking such ROS to the carcinogenicity observed in rodents treated with certain peroxisome proliferators.
Ye 1995	Chromium (Cr)	The activation of NF- κ B, which may be involved in the mechanism of Cr(VI)-induced carcinogenicity, was decreased by a metal chelator, DTPA or catalase, but increased by SOD. ESR measurements using DMPO confirmed that the incubation of Cr(VI) with the Jurkat cells in the presence of glutathione reductase generated hydroxyl radicals.
Toyokuni 1996	Iron (Fe)	Fe-induced oxidative stress results in two possible consequences: (1) redox regulation failure that leads to lipid peroxidation and oxidative DNA and protein damage; (2) redox regulation that activates a variety of reducing and oxystress-protective mechanisms. Both consequences appear to play a role in Fe-induced carcinogenesis.
Liu 1997	Chromium (Cr)	Cr(VI)-glutathione complex is able to generate hydroxyl radicals via a Fenton-like reaction in the presence of molecular oxygen in aqueous medium, implying the possible involvement of Cr(IV)-mediated production of hydroxyl radicals in the mechanism of Cr(VI)-induced carcinogenesis.
Ding* 1999	Vanadium	SOD and catalase but not sodium formate inhibited vanadate-induced AP-1 activation, which might be a causative factor of the carcinogenicity of vanadium, in mouse epidermal JB6P1 cells, suggesting that superoxide anion and hydrogen peroxide, but not hydroxyl radicals are involved in the activation.
Hoyo 2000	Chromium (Cr)	<i>In vivo</i> hydroxyl radical generation in blood of mice after acute Cr(VI) intake was detected by applying ESR spin-trapping technique.
Shi 2001	Arsenic (As)	Superoxide anion and hydrogen peroxide were detected in keratinocytes incubated with arsenite [As(III)] concomitant with DNA damage. Since sodium formate and deferoxamine reduced the DNA damage, exposure to As(III) generates superoxide anions and hydrogen peroxide, and hydroxyl radical, derived from hydrogen peroxide, may play an important role in As-induced skin carcinogenicity.
Abe 2008	Copper (Cu)	Copper gluconate possesses carcinogenic risk toward the liver at the high dose level, and that oxidative stress and inflammatory and pro-apoptotic signaling statuses may participate in its underlying mechanisms.

*The study by Ding *et al.* was skeptical about the involvement of hydroxyl radicals in the vanadium-induced carcinogenicity.

mediated by SOD. The resultant hydrogen peroxide is then converted to the hydroxyl radical via a Fenton-like reaction. The hydroxyl radical leads to DNA damage and may activate redox-sensitive transcription factors such as NF- κ B and AP-1 that have been shown to play important roles in tumor promotion.⁽⁴⁰⁻⁴⁴⁾

As described above, DNA lesions can be caused by mutagenic/carcinogenic agents. In general, a repair pathway is activated in response to such agents to preserve the integrity of the genome,⁽⁴⁵⁻⁴⁹⁾ and the activation of cellular responses ranging from DNA repair and chromatin remodeling to the activation of apoptosis occurs if the damage is irreparable.⁽⁵⁰⁻⁵²⁾ Besides the involvement of oxidative stress in metal (Cr(VI) and arsenic)-induced carcinogenicity, the cocarcinogenic and comutagenic effects of metals likely stem from their ability to interfere with DNA repair processes via DNA methylation and histone modifications,^(53,54) suggesting that hydroxyl radical is one of the causative factors of metal-induced carcinogenicity (Fig. 2).

De Flora commented that the carcinogenicity of Cr(VI) requires massive exposure, as is only encountered in well-defined occupational settings, and is site specific, being specifically targeted to the lung and, in some cases, to the sinonasal cavity.⁽⁵⁵⁾ As reviewed by Smith *et al.*,⁽⁵⁶⁾ arsenic can cause liver, lung, kidney, and bladder cancer, and the population cancer risk due to arsenic in US water supplies may be comparable to that due to environmental tobacco smoke and radon in homes. In 2008, the National Toxicology Program completed a 2-year cancer bioassay for Cr(VI) in drinking water using mice. Investigators found that Cr(VI) caused tumors in the small intestines of mice and the oral mucosa of rats at 20–180 mg/l Cr(VI) in the form of sodium dichromate dihydrate.⁽⁵⁷⁾ Cui *et al.*⁽⁵⁸⁾ reported that chronic oral exposure of mice to inorganic arsenate(V) in drinking water for 18 months induced lung cancer. These results support the notion that long-term exposure of cells to heavy metals (Cr(VI) and arsenic) is required to cause carcinogenesis, even though the hydroxyl radical is a causative factor.

There are also potential advantages to the use of the hydroxyl

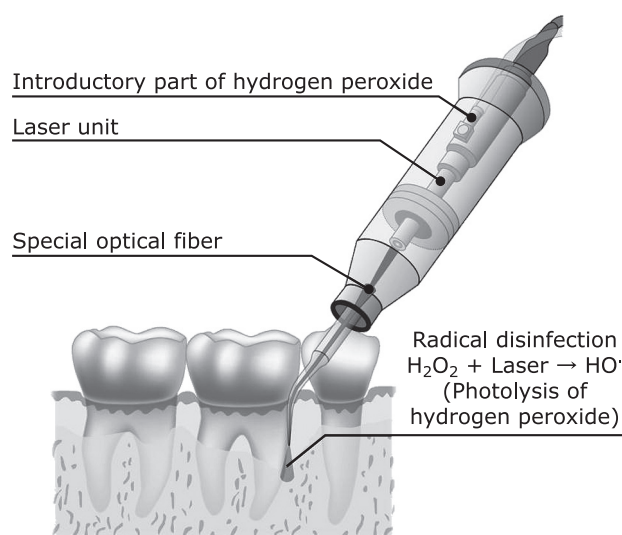


Fig. 1. Schematic illustration of a device for the treatment of dental infectious diseases utilizing photolysis of H₂O₂. The photolysis of H₂O₂ system is incorporated into conventional ultrasound scaler.

radical, being included in host immune defense utilizing radical by PMNs,^(59,60) and medical applications such as for cancer treatment and antibiotics.^(61,62) As an example, Kohanski reported that all three major classes of bactericidal drugs (quinolones, β -lactams, and aminoglycosides) utilize a common mechanism of killing whereby they stimulate the production of lethal amounts of hydroxyl radicals via the Fenton reaction.⁽⁶²⁾ Interestingly, Wlassoff reported a new approach to the treatment of cancer based on the innate overproduction of hydrogen peroxide in cancer cells.⁽⁶³⁾ Hydrogen peroxide serves as a prodrug in the presence of transi-

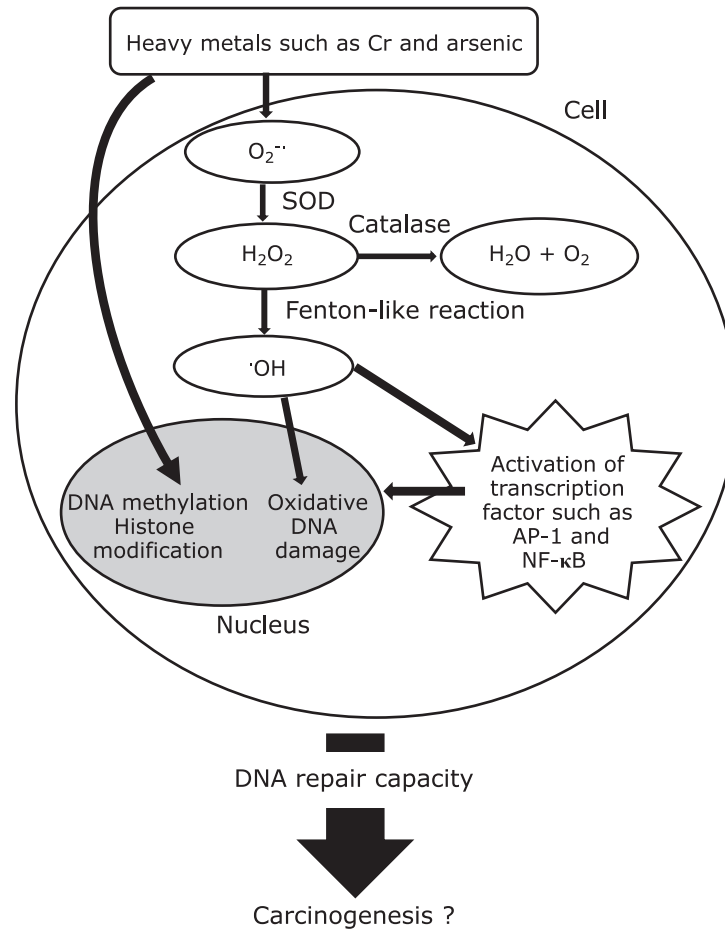


Fig. 2. Schematic drawing of the possible mechanism generating hydroxyl radicals and its role in heavy metal-mediated carcinogenicity.

tion metal ions, such as iron delivered by ferrocene ($\text{Fe}(\text{C}_5\text{H}_5)_2$). Under the effect of ferrocene, hydrogen peroxide is split into hydroxyl anions and hydroxyl radicals, the latter of which induces apoptosis, leading to elimination of cancer cells. The hydroxyl radical is also free from residual toxicity because of its extremely short-half life, approximately 10^{-9} s.^(7,8) In addition, hydrogen peroxide, which was reported to be mutagenic and an intermediate able to generate hydroxyl radicals via a Fenton-like reaction in Cr- and arsenic-induced carcinogenicity,^(12,16,28-30) is used in medical treatment for disinfection. Therefore, it is concluded that there is little to no risk of carcinogenicity as long as the hydroxyl radical is used as a disinfectant for short-term treatment of the oral cavity as reported in our previous study.⁽¹⁾

Abbreviations

AP-1	activator protein-1
Cr	chromium
DMPO	5,5-dimethyl-1-pyrroline- <i>N</i> -oxide
DMSO	dimethyl sulfoxide
ESR	electron spin resonance
F-12	fecapentaene-12
NF-κB	nuclear factor-κB
p-AP	p-aminophenol
PMA	phorbol-12-acetate-13-myristate
PMN	polymorphonuclear leukocyte
ROS	reactive oxygen species
SOD	superoxide dismutase

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