Influence of chlorine dioxide on cell death and cell cycle of human gingival fibroblasts

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ABSTRACT

Objectives: The effects of chlorine dioxide (ClO_2), sodium hypochlorite (NaOCl), and hydrogen peroxide (H_2O_2) on cell death and the cell cycle of human gingival fibroblast (HGF) cells were examined.

Methods: The inhibition of HGF cell growth was evaluated using a Cell Counting Kit-8. The cell cycle was assessed with propidium iodide-stained cells (distribution of cells in G_0/G_1, S, G_2/M phases) using flow cytometry. The patterns of cell death (necrosis and ap optosis) were analyzed using flow cytometry with annexin V-FITC/PI staining.

Results: The lethal doses for 50% of the cells (LD_{50}) of ClO_2, NaOCl, and H_2O_2 were 0. 16 mM, 0.79 mM, and 0.11 mM, respectively. All three dental disinfectants induced G_0/G_1 cell cycle arrest. H_2O_2 induced apoptosis at concentrations of 0.05 and 0.1 mM, while NaOCl and ClO_2 did not induce significant apoptosis at any concentration examined.

Conclusions: These results suggest that ClO_2 is sufficient for use as a dental disinfectant compared with H_2O_2 or NaOCl.

1. Introduction

Various disinfectants are used in endodontics and for gingival and soft tissue irrigation. Disinfectants are administered to the oral mucosa or to the root canals of pulpless teeth directly.[1–3] These disinfectants can be absorbed into the bloodstream through the oral mucosa or by passing through the apical foramen of teeth.[4–6] Consequently, tissue cytotoxicity and biocompatibility must be considered when choosing a disinfectant.

Sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2) are widely used as endodontic irrigants.[7–11] However, H_2O_2 and NaOCl have deleterious effects on cell DNA, and have been implicated in several pathological processes, such as cancer.[12–14]

Chlorine dioxide (ClO_2) is currently used as a water purifier, surface disinfectant, and in some commercial mouth rinses.[15–18] ClO_2 has bactericidal activity and is toxic to both enveloped and non-enveloped viruses.[19, 20] It has been considered for use as a root canal irrigant, but little is known about its cytotoxicity.

This study was performed to evaluate the cytotoxicity of aqueous solutions of ClO_2 on human gingival fibroblast (HGF) cells in comparison with H_2O_2 and NaOCl, and to compare the effects of these disinfectants on the HGF cell cycle and cell death.

2. Materials and Methods

2.1. Chemicals

This study used the chemicals 30% H_2O_2 (Santoku Chemical Industries, Tokyo, Japan), 10% NaOCl (Sigma-Aldrich, Tokyo, Japan) solution, and aqueous ClO_2 (Kurosaki, Fukuoka, Japan). The aqueous ClO_2 solution was prepared by mixing solution A (chiefly $NaOCl_2$) and solution B (chiefly HCl), resulting in a concentration of 0.3% (45 mM) after 1 h. We diluted samples of each stock solution to the appropriate final concentration using Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Daiichi Pure Chemicals, Tokyo, Japan) immediately before use.

2.2. Cells and cell culture

Human gingival fibroblasts were cultivated from gingiva obtained from a patient's tooth after obtaining informed consent according to institutional committee regulations. We incubated the cells in culture medium consisting of DMEM, 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2.

2.3. Growth assay

We used a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) to determine the lethal dose

50% (LD_{50}). We seeded an equal number of HGF cells (1×10^4 cells/well) in 100 µl of DMEM into 96-well microplates and incubated the cells overnight at 37°C in a humidified atmosphere of 5% CO_2. We prepared a stock solution of each disinfectant and then diluted each solution serially in the cell culture medium. Then, we treated the cells with each disinfectant diluted in 100 µl of DMEM to obtain final concentrations of 10^{-0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} mM.

Following incubation for 24 h, 10 µl of Cell Counting Kit-8 solution was added to each well and the plates were incubated for an additional 2 h at 37°C. We measured the absorbance at 450 nm with an immunoreader (Microplate Reader 550; Bio-Rad, Tokyo, Japan) and calculated the number of cells from the absorbance value relative to a standard curve.

2.4. Cell cycle analysis

We plated 5×10^5 HGF cells in 60-mm culture dishes and incubated them in DMEM with 0.5% FBS for 48 h to synchronize the cells in G_0/G_1 phase by serum deprivation.[21] We induced the cells to re-enter the cell cycle by incubation in fresh medium with the disinfectants at the final concentration (determined from the growth assay results) for a further 24 h.

The cellular DNA content was determined by flow cytometry, as described previously.[22] We collected the floating and attached cells using trypsin-EDTA and resuspended them in DMEM (no FBS). The cells were fixed for 30 min in an ice-cold 70% ethanol solution containing ribonuclease (RNase; 2 mg/ml). We washed the cells in PBS, and then stained them with propidium iodide (PI) for 10 min. The PI-elicited fluorescence was measured for individual cells using a flow cytometer (FACSCalibur; Becton Dickinson, Tokyo, Japan) with laser excitation at 488 nm. Emissions greater than 590 nm were collected in a linear/log scale fashion. We analyzed a total of 1×10^4 cells for each sample and determined the percentages of cells in G_0/G_1, S, and G_2/M phases using standard ModiFit and Cell Quest software (Becton Dickinson).

2.5. Annexin V-FITC/PI assay

To detect apoptosis, the cells were stained with PI and fluorescein isothiocyanate (FITC)-conjugated annexin V using an Annexin V-FITC Apoptosis Detection Kit I (Becton Dickinson). Annexin V-FITC identifies cells in early apoptosis by detecting externalized phosphatidylserine, and PI identifies cells that have lost plasma membrane integrity (*i.e.*, necrotic or late apoptotic cells). We added the disinfectant concentration that previously produced growth in the HGF cultures and incubated the cells for 24 h at

37°C. After incubation, the cells were harvested by trypsin-EDTA treatment and washed with DMEM. We resuspended the cells in 50 μl of 1× binding buffer supplemented with 5 μl of annexin V-FITC and 10 μl of PI, and kept the cells at room temperature in the dark for 15 min according to the manufacturer's instructions. Following the addition of 450 μl of 1× binding buffer, the stained cells were kept on ice and subjected to fluorescence-activated cell sorter analysis (FACS) using a FACSCalibur flow cytometer. We measured the FITC fluorescence between 515 and 545 nm and the PI fluorescence between 564 and 606 nm.

2.6. Data analysis

The data are expressed as the means ± standard error (SE) of at least three independent experiments; they were analyzed using the Student's *t* test; $p < 0.05$ was considered significant.

3. Results

3.1. Growth assay

We expressed cell proliferation as the percentage of cells that proliferated in the presence of a disinfectant relative to proliferation in its absence. The LD_{50} for HGF cells was 1.6×10^{-1} mM for ClO_2, 1.1×10^{-1} mM for H_2O_2, and 7.9×10^{-1} mM for NaOCl (Fig. 1).

3.2. Cell cycle analysis

Serum-deprived cells were arrested in G_0/G_1 phase (Fig. 2; control 0 h). When these cells were returned to culture medium containing 10% FBS, cell proliferation was stimulated after 24 hours and the number of cells in S and G_2/M phases increased and the number in G_0/G_1 phase decreased, compared with control 0 h (Fig. 2; control 24 h). By contrast, the cells exposed to the disinfectants caused a large percentage of cells to remain in G_0/G_1 phase, with a small percentage in S and G_2/M phases (Fig. 3).

3.3. Annexin V-FITC/PI assay

Figure 4 shows the distribution of the cell populations after treatment with disinfectants for 24 h. A dot-plot of annexin V-FITC vs. PI showed four separate clusters: viable cells (lower left quadrant), early apoptotic cells (lower right quadrant), necrotic or late

apoptotic cells (upper right quadrant), and necrotic cells (upper left quadrant). The Student's *t* test revealed that H_2O_2 (0.05 and 0.1 mM) induced significantly more apoptosis compared to the control 24 h ($p < 0.05$), while ClO_2 and NaOCl did not produce significant levels of apoptosis (Fig. 5).

4. Discussion

NaOCl and H_2O_2 are widely used as endodontic disinfectants. NaOCl, which is used at a concentration of 5.25% (7.8×10^2 mM), was shown to have both antibacterial and necrotic tissue dissolution properties.[8, 9,23] H_2O_2 is used as a cleansing agent in wound debridement and as an intracanal disinfectant at concentrations of 2.5–3.5% (7.4×10^2 to 1.0×10^3 mM).[1]

Chlorine dioxide (ClO_2) is currently used as a water purifier, surface disinfectant, and in some commercial mouth rinses.[15–18] ClO_2 has recently been considered as a root canal irrigant because of its antibacterial activity and compatibility with living tissue,[19] but at present, little is known about its cytotoxicity.

ClO_2 is used as a disinfectant at concentrations of 0.1–15 mM,[15, 24, 25] but an effective clinical concentration has not been determined. If a lower effective concentration can be determined, ClO_2 may prove to be a relatively nontoxic disinfectant.

In this study, the LD_{50} for HGF cells at 24 h was found to be 1.6×10^{-1} mM for ClO_2, 1.1×10^{-1} mM for H_2O_2, and 7.9×10^{-1} mM for NaOCl (Fig. 1). The results of the cell cycle analysis suggested that at the LD_{50}, the HGF cells were arrested in G_0/G_1 phase (Fig. 3). This implies that disinfectant inhibition of cell growth was due to the arrest of DNA replication in the cell cycle.

The inhibition of cell growth causes cell death either through necrosis (*i.e.*, accidental cell death) or apoptosis (*i.e.*, programmed cell death).[26-28] The morphological changes associated with necrosis are swelling, membrane rupture, and cell lysis. In contrast, during apoptosis, condensation and fragmentation of the cytoplasm and nucleus occur in the target cells, while the normal organelle structures are maintained. Several studies have indicated that exposing cells to exogenous oxidants can induce apoptosis.[29]

During the early stages of apoptosis, a loss of phospholipid asymmetry occurs that results in the translocation of negatively charged aminophospholipid phosphatidylserine (PS) from the inner to the outer leaflet of the cell membrane.[30] The annexin V-FITC/PI assay used to identify apoptotic cells detects this change as a marker of apoptotic cells. In the presence of calcium ions, annexin V binds specifically to PS, and the externalization of PS is an important cell-surface signal for the macrophage clearance of apoptotic cells.[31, 32]

Apoptosis is a physiological process involved in cell deletion during organogenesis, and it controls cell proliferation and differentiation in adult tissues.[33] The deregulation of apoptosis has been implicated in a variety of diseases. Excessive apoptosis has been related to sepsis, ischemic heart disease, stroke, and neurodegenerative diseases, while

functional deficiencies in the activation of apoptosis may facilitate the development of cancer and autoimmune diseases.[34]

We examined the effects of disinfectants on cell death using an annexin V-FITC/PI assay at close to the LD_{50} concentration (0.05, 0.1, and 0.5 mM). The dose-independent effect showed that H_2O_2, but not ClO_2 or NaOCl, induced cell death by producing an apoptotic signal at 0.05 and 0.1 mM (Fig. 4, 5). We demonstrated that these disinfectants consistently arrested cells at G_0/G_1 phase of the cell cycle and induced apoptosis and necrosis. H_2O_2 arrested cells at G_0/G_1 phase of the cell cycle and induced apoptosis. ClO_2 and NaOCl arrested cells at G_0/G_1 phase of the cell cycle, however, did not induce apoptosis. In summary, ClO_2 and NaOCl may be better dental disinfectants than oxidative agents, such as H_2O_2.

Chlorinating agents, such as Cl_2 and NaOCl, used as disinfectants for drinking water react with natural organic matter to produce halogenated disinfectant by-products. Trihalomethanes and haloacetic acids are the two most prevalent groups of disinfectant by-products formed during the chlorination of natural water.[35] A recent study showed that trihalomethane is an animal carcinogen and a suspected human carcinogen.[36] ClO_2 produces little or no trihalomethanes,[37] and may be a better dental disinfectant than NaOCl.

These findings suggest that ClO_2 is sufficient for use as a dental disinfectant compared with H_2O_2 or NaOCl. Further *in vitro* and *in vivo* studies are required to determine the clinical relevance of our findings.

References

1. Hori I, Higo Y, Ohno M, Tsutsui TW, Tsutsui T. Assessment using human dental pulp cells of clastogenicity of antiseptics used in dental practice and agents for root canal enlargement and cleaning. *Odontology / the Society of the Nippon Dental University* 2007; **95**:30-7.

2. Ari H, Erdemir A, Belli S. Evaluation of the effect of endodontic irrigation solutions on the microhardness and the roughness of root canal dentin. *Journal of endodontics* 2004; **30**:792-5.

3. Siqueira JF Jr, Magalhães KM, Rôças IN. Bacterial reduction in infected root canals treated with 2.5% NaOCl as an irrigant and calcium hydroxide/camphorated paramonochlorophenol paste as an intracanal dressing. *Journal of endodontics* 2007; **33**:667-72.

4. Myers DR, Shoaf HK, Dirksen TR, Pashley DH, Whitford GM, Reynolds KE. Distribution of 14C-formaldehyde after pulpotomy with formocresol. *Journal of the American Dental Association* 1978; **96**:805-13.

5. Bartelstone HJ. Radioiodine penetration through intact enamel with uptake by bloodstream and thyroid gland. *Journal of dental research* 1951; **30**:728-33

6. Someya H, Higo Y, Ohno M, Tsutsui TW, Tsutsui T. Clastogenic activity of seven

endodontic medications used in dental practice in human dental pulp cells. *Mutation research* 2008; **650**:39-47.

7. Whitten BH, Gardiner DL, Jeansonne BG, Lemon RR. Current trends in endodontic treatment: report of a national survey. *Journal of the American Dental Association* 1996; **127**:1333-41.

8. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. *International endodontic journal* 2001; **34**:424-8.

9. Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. *Journal of endodontics* 1978; **4**:60-4.

10. Qing Y, Akita Y, Kawano S, Kawazu S, Yoshida T, Sekine I. Cleaning efficacy and dentin micro-hardness after root canal irrigation with a strong acid electrolytic water. *Journal of endodontics* 2006; **32**:1102-6.

11. Erdemir A, Ari H, Güngüneş H, Belli S. Effect of medications for root canal treatment on bonding to root canal dentin. *Journal of endodontics* 2004; **30**:113-6.

12. Ohnishi S, Murata M, Kawanishi S. DNA damage induced by hypochlorite and hypobromite with reference to inflammation-associated carcinogenesis. *Cancer Lett*

2002; **178**:37-42.

13. López-Lázaro M. Dual role of hydrogen peroxide in cancer: possible relevance to cancer chemoprevention and therapy. *Cancer Lett* 2007; **252**:1-8.

14. Daroui P, Desai SD, Li TK, Liu AA, Liu LF. Hydrogen peroxide induces topoisomerase I-mediated DNA damage and cell death. *J Biol Chem* 2004; **279**:14587-94.

15. Wirthlin MR, Marshall GW JR. Evaluation of ultrasonic scaling unit waterline contamination after use of chlorine dioxide mouthrinse lavage. *Journal of periodontology* 2001; **72**:401-10.

16. Wirthlin MR, Marshall GW JR, Rowland RW. Formation and decontamination of biofilms in dental unit waterlines. *Journal of periodontology* 2003; **74**:1595-609.

17. Zani C, Feretti D, Buschini A, Poli P, Rossi C, Guzzella L, Caterino FD, Monarca S. Toxicity and genotoxicity of surface water before and after various potabilization steps. *Mutation research* 2005; **587**:26-37.

18. Peruzzo DC, Jandiroba PF, Nogueira Filho Gda R. Use of 0.1% chlorine dioxide to inhibit the formation of morning volatile sulphur compounds (VSC). *Braz Oral Res* 2007; **21**:70-4.

19. Barnhart BD, Chuang A, Lucca JJ, Roberts S, Liewehr F, Joyce AP. An in vitro evaluation of the cytotoxicity of various endodontic irrigants on human gingival

fibroblasts. *Journal of endodontics* 2005; **31**:613-5.

20. Zoni R, Zanelli R, Riboldi E, Bigliardi L, Sansebastiano G. Investigation on virucidal activity of chlorine dioxide. experimental data on feline calicivirus, HAV and Coxsackie B5. *Journal of preventive medicine and hygiene* 2007; **48**:91-5.

21. Kühholzer B, Prather RS. Synchronization of porcine fetal fibroblast cells with topoisomerase-inhibitor hoechst 33342. *Animal reproduction science* 2001; **66**:109-16.

22. Otsubo A, Bhawal UK, Nomura Y, Mitani Y, Ozawa K, Kuniyasu H, Sugiyama M. UCN-01 (7-hydroxystaurosporine) induces apoptosis and G1 arrest of both primary and metastatic oral cancer cell lines *in vitro*. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics* 2007; **103**:391-7.

23. Shih M, Marshall FJ, Rosen S. The bacterial efficiency of sodium hypochlorite as an endodontic irrigant. *Oral surgery, oral medicine, and oral pathology* 1970; **29**:613-9.

24. Li JW, Xin ZT, Wang XW, Zheng JL, Chao FH. Mechanisms of inactivation of hepatitis A virus in water by chlorine dioxide. *Water research* 2004; **38**:1514-9.

25. Isomoto H, Urata M, Kawazoe K, Matsuda J, Nishi Y, Wada A, Ohnita K, Hirakata Y, Matsuo N, Inoue K, Hirayama T, Kamihira S, Kohno S. Endoscope disinfection using chlorine dioxide in an automated washer- disinfector. *The Journal of hospital infection* 2006; **63**:298-305.

26. Yonish-Rouach E, Grunwald D, Wilder S, Kimchi A, May E, Lawrence JJ, May P, Oren M. p53-mediated cell death: relationship to cell cycle control. *Molecular and cellular biology* 1993; **13**:1415-23.

27. Liu L, Trimarchi JR, Keefe DL. Thiol oxidation-induced embryonic cell death in mice is prevented by the antioxidant dithiothreitol. *Biology of reproduction* 1999; **61**:1162-9.

28. Mazière C, Meignotte A, Dantin F, Conte MA, Mazière JC. Oxidized LDL induces an oxidative stress and activates the tumor suppressor p53 in MRC5 human fibroblasts. *Biochem Biophys Res Commun* 2000; **276**:718-23.

29. Chen HY, Zheng CY, Zou GL, Xie DX, Gong JP. Peplomycin induces G1-phase specific apoptosis in liver carcinoma cell line Bel-7402 involving G2-phase arrest. *Acta pharmacologica Sinica* 2004; **25**:1698-704

30. Maulik N, Kagan VE, Tyurin VA, Das DK. Redistribution of phosphatidylethanolamine and phosphatidylserine precedes reperfusion-induced apoptosis. *The American journal of physiology* 1998; **274**:H242-8.

31. Masuki K, Nomura Y, Bhawal UK, Sawajiri M, Hirata I, Nahara Y, Okazaki M. Apoptotic and necrotic influence of dental resin polymerization initiators in human gingival fibroblast cultures. *Dental materials journal* 2007; **26**:861-9.

32. Kagan VE, Gleiss B, Tyurina YY, Tyurin VA, Elenström-Magnusson C, Liu SX, Serinkan FB, Arroyo A, Chandra J, Orrenius S, Fadeel B. A role for oxidative stress in apoptosis: oxidation and externalization of phosphatidylserine is required for macrophage clearance of cells undergoing Fas-mediated apoptosis. *Journal of immunology* 2002; **169**:487-99.

33. Yuan J, Murrell GA, Trickett A, Wang MX. Involvement of cytochrome c release and caspase-3 activation in the oxidative stress-induced apoptosis in human tendon fibroblasts. *Biochimica et biophysica acta* 2003; **1641**:35-41.

34. Formichi P, Radi E, Battisti C, Tarquini E, Leonini A, Di Stefano A, Federico A. Human fibroblasts undergo oxidative stress-induced apoptosis without internucleosomal DNA fragmentation. *Journal of cellular physiology* 2006; **208**:289-97.

35. Aggazzotti G, Fantuzzi G, Righi E, Predieri G. Environmental and biological monitoring of chloroform in indoor swimming pools. *Journal of chromatography* 1995; **710**:181-90.

36. Lévesque B, Ayotte P, Tardif R, Ferron L, Gingras S, Schlouch E, Gingras G, Levallois P, Dewailly E. Cancer risk associated with household exposure to chloroform. *Journal of toxicology and environmental health Part A* 2002; **65**:489-502.

37. Hua G, Reckhow DA. Comparison of disinfection byproduct formation from chlorine and alternative disinfectants. *Water research* 2007; **41**:1667-78.

Figure captions

Fig. **1**. Dose-related cytotoxicity following 24-h exposure to the disinfectants. Cells (10^4 cells) in 96-well culture plates were exposed to different concentrations of disinfectant for 24 h. The LD_{50} concentration of each reagent is indicated by the intersection of the plotted line.

Fig. **2**. **A**: The representative flow cytometry profile of HGF cells at 0 and 24 h in the control condition. The high peak (left) indicates cells in G_0/G_1 phase, the smaller peaks (right) represent cells in G_2/M phase and the area between the peaks represents the cells in S phase. **B**: The histogram representing percentage of cells in each cell cycle phase at 0 and 24 h under the control condition.

Fig. **3**. The percentage of cells in G_0/G_1, S, and G_2/M phases. Cultures exposed to NaOCl, ClO_2, and H_2O_2 have significantly more cells in G_0/G_1 phase compared to the control. *Significantly different from the control at 24 h.

Fig. **4**. The FACS analyses after staining with annexin V-FITC and PI show the induction

of apoptosis and necrosis in HGF cells after a 24-h exposure to the disinfectants. In each plot, the lower left quadrant (LL) represents viable cells, the upper left quadrant (UL) indicates necrotic cells, the lower right quadrant (LR) denotes early apoptotic cells, and the upper right quadrant (UR) represents necrotic or late apoptotic cells.

Fig. **5**. The FACS statistical analysis for each disinfectant. The only significant difference found was an increase in apoptotic cells (LR) induced by H_2O_2 at 0.05 mM and 0.1 mM (*$p < 0.05$). The lower left (LL) shows viable cells; the upper left (UL) shows necrotic cells; the lower right (LR) shows early apoptotic cells; and the upper right (UR) shows necrotic or late apoptotic cells.

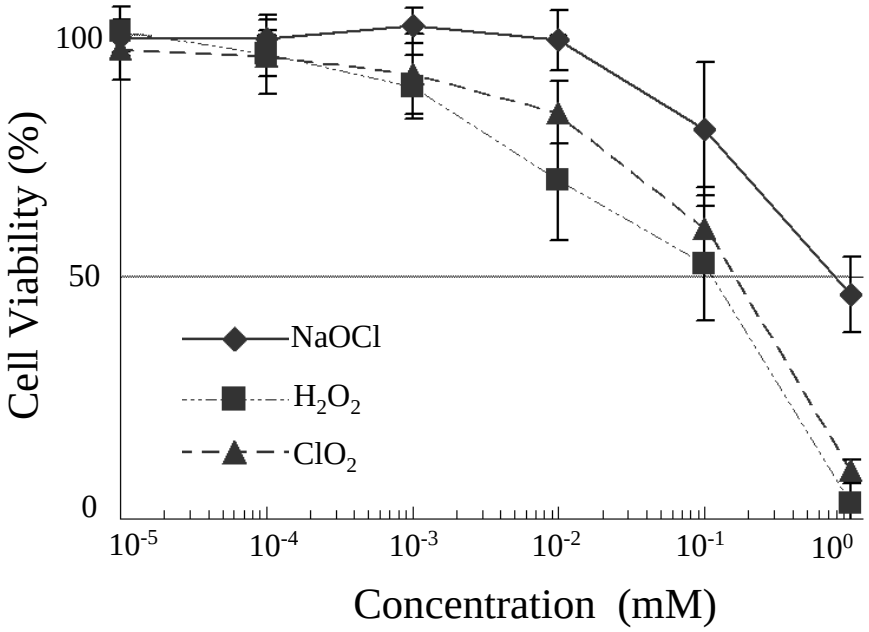
100
50
0
Cell Viability (%)
NaOCl
H_2O_2
ClO_2
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
Concentration (mM)

Fig. 1

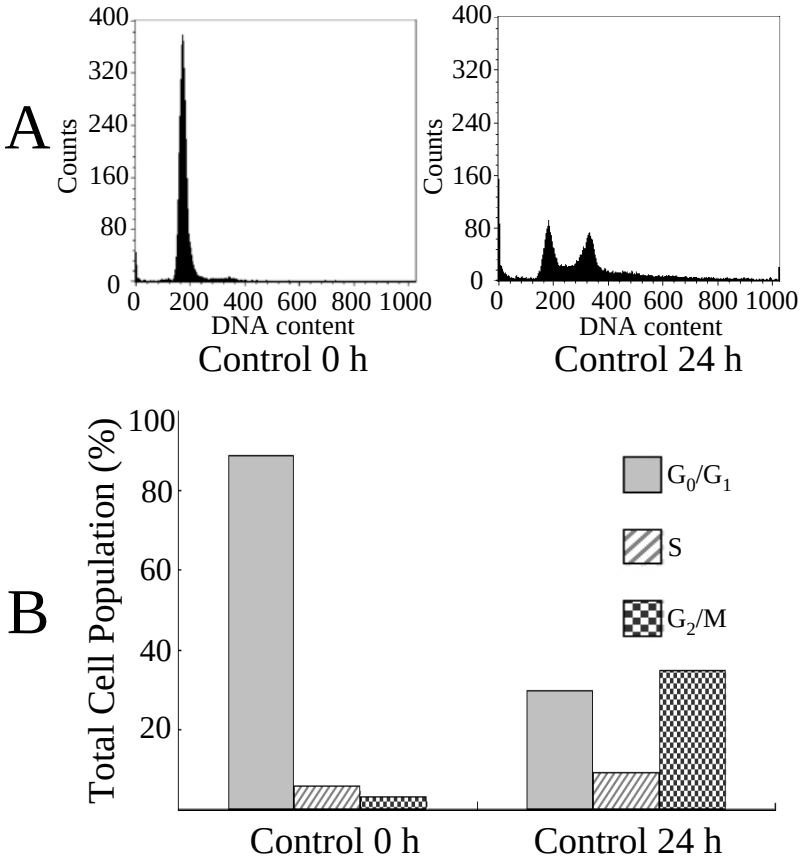
A
Counts
400
320
240
160
80
0
0 200 400 600 800 1000
DNA content
Control 0 h
Control 24 h
B
Total Cell Population (%)
100
80
60
40
20
G_0/G_1
S
G_2/M
Control 0 h
Control 24 h

Fig 2

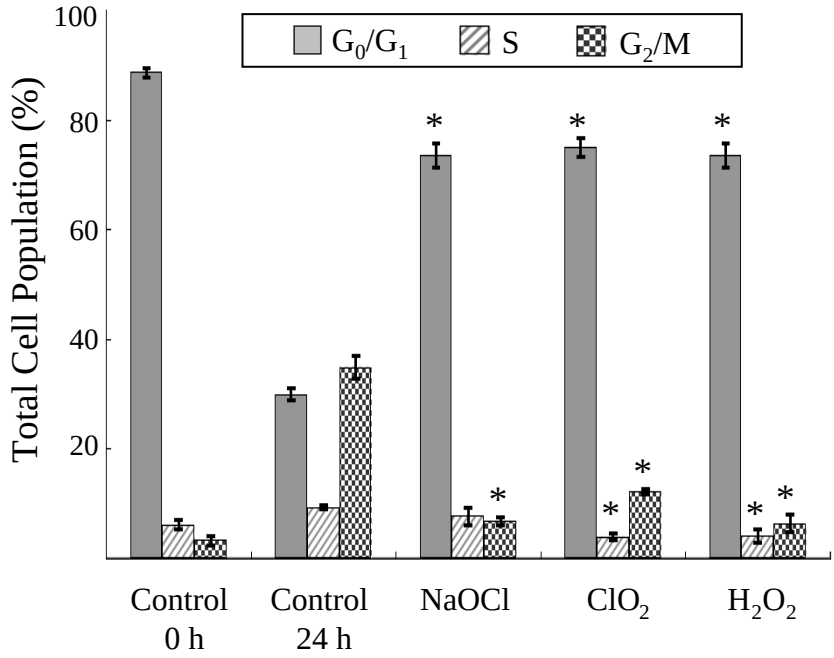
100
80
60
40
20
Total Cell Population (%)
G0/G1
S
G2/M
*
Control 0 h
Control 24 h
NaOCl
ClO2
H2O2

Fig. 3

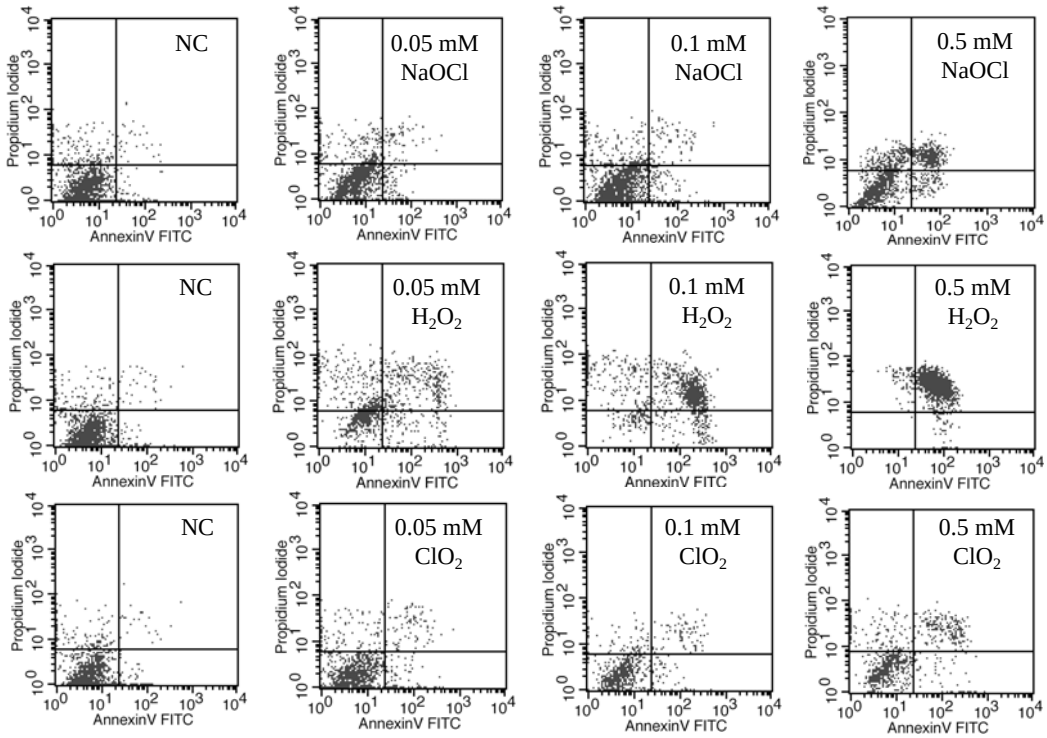
NC
0.05 mM NaOCl
0.1 mM NaOCl
0.5 mM NaOCl
NC
0.05 mM H_2O_2
0.1 mM H_2O_2
0.5 mM H_2O_2
NC
0.05 mM ClO_2
0.1 mM ClO_2
0.5 mM ClO_2
Propidium Iodide
AnnexinV FITC

Fig. 4

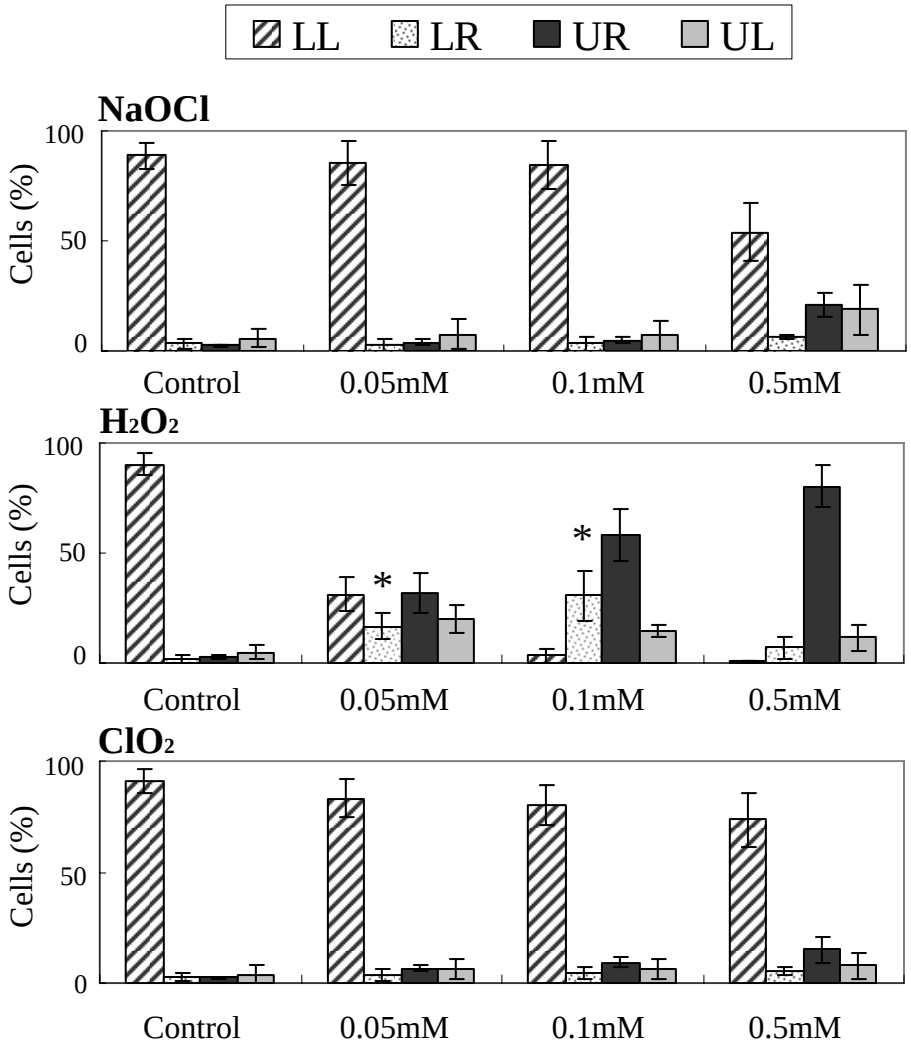
LL
LR
UR
UL
NaOCl
Cells (%)
100
50
0
Control
0.05mM
0.1mM
0.5mM
H2O2
Cells (%)
100
50
0
*
*
Control
0.05mM
0.1mM
0.5mM
ClO2
Cells (%)
100
50
0
Control
0.05mM
0.1mM
0.5mM

Fig 5