

# The Anti-Inflammatory Mechanism of 635 nm Light-Emitting-Diode Irradiation Compared With Existing COX Inhibitors

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**Background and Objectives:** Inhibition of cyclooxygenase (COX) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) protects cells against cell injury in specific pathophysiological situations: inflammation and oxidative stress. Although the anti-inflammatory effects have been reported in clinical fields for specific wavelength irradiation during wound healing, the physiological mechanism has not been clarified yet. The aim of the present study is to investigate the anti-inflammatory mechanism of 635 nm light-emitting-diode (LED) irradiation compared with existing COX inhibitors.

**Study Design/Materials and Methods:** The present study investigated anti-inflammatory effects of 635 nm irradiation on PGE<sub>2</sub> release, COX and phospholipase A<sub>2</sub> (PLA<sub>2</sub>) expression, and reactive oxygen species (ROS) dissociation in arachidonic acid (AA)-treated human gingival fibroblast (hGF). These results were compared with their existing COX inhibitors: indomethacin and ibuprofen. The PGE<sub>2</sub> release was measured by enzyme immunoassay, the COX expression was measured by western blot and reverse transcriptase polymerase chain reaction (RT-PCR), and ROS level was measured by flow cytometry, laser scanning confocal microscope and RT-PCR.

**Results:** Results showed that 635 nm irradiation and existing COX inhibitors inhibit expression of COX and PGE<sub>2</sub> release. Unlike indomethacin and ibuprofen, 635 nm irradiation leads to a decrease of ROS levels and mRNA expression of cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) and secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>).

**Conclusion:** Taken together, 635 nm irradiation, unlike indomethacin and ibuprofen, can directly dissociate the ROS. This inhibits cPLA<sub>2</sub>, sPLA<sub>2</sub>, and COX expression, and results in the inhibition of PGE<sub>2</sub> release. Thus, we suggest that 635 nm irradiation inhibits PGE<sub>2</sub> synthesis like COX inhibitor and appears to be useful as an anti-inflammatory tool. *Lasers Surg. Med.* 39:614–621, 2007. © 2007 Wiley-Liss, Inc.

**Key words:** inflammation; PGE<sub>2</sub>; indomethacin; ibuprofen; LED; photodissociation; reactive oxygen species; arachidonic acid

## INTRODUCTION

Cyclooxygenase (COX) inhibitors bind to the COX active site to inhibit prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) synthesis,

which is known to be a central role of inflammation [1]. A variety of COX inhibitors are used in anti-inflammatory drugs to inhibit the conversion of arachidonic acid (AA) to prostaglandin E<sub>2</sub> by COX. COX is known to exist in two isoforms: COX-1 and COX-2. COX-1, a constitutive isoenzyme, influences the maintenance of physiological functions such as platelet aggregation, cytoprotection in the stomach, and maintenance of normal kidney functions [2,3]. COX-2 has been shown to be induced by proinflammatory cytokines, endotoxins, mitogens, and tumor promoters at the inflammatory site [4,5]. Inhibition of this COX-1 or COX-2 enzyme by non-steroidal anti-inflammatory drugs (NSAID) such as aspirin, indomethacin, and ibuprofen allow the level of prostaglandins (PGs) to reduce, resulting in a reduction in pain and inflammation. Especially, selective COX-2 inhibitors such as ibuprofen or NS-398 have reduced gastrointestinal liability [1,2,6].

Recently, many reports have suggested that a specific wavelength of light irradiation could be an alternative anti-inflammatory tool for wound healing in clinical fields [7,8]. Many physiological clinical results of low-level laser treatment (LLLT) for pain reduction, anti-inflammation, and acceleration of wound healing have been reported [9–11]. Also, it has been reported that low-level laser irradiation inhibits PGE<sub>2</sub> production and mRNA expression of COX-1 and COX-2 [12]. Especially, the 632.8 nm wavelength of He–Ne laser was recommended [9,10]. This 632.8 nm wavelength of irradiation can improve the electron transfer of cytochrome *c* oxidase in mitochondria, and then increase ATP synthesis and cell metabolism [13,14]. A recent report has shown that a similar wavelength of a light-emitting-diode (LED) instead of a laser instrument as a light source has similar biological effects, but little is known about the mechanism of light irradiation on anti-inflammation [8]. In living organisms, oxygen radical or reactive oxygen species

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(ROS) are ubiquitous and occur naturally in all aerobic species, coming from exogenous and endogenous sources. Intracellular ROS have been regarded as a critical factor in various kinds of inflammation [15,16]. The remarkable effects of ROS during inflammation are the oxidative modification of phospholipids through phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activation within the cell membrane and stimulation of the mRNA expression of COX-2 [17,18]. Light irradiation itself has been known to be able to work as an electron transition in some molecules, as elevated electrons induce a decrease from higher energy levels to lower energy levels [19–21]. A ROS such as superoxide (O<sub>2</sub><sup>-</sup>) can be reduced in the occurring electron transition by visible light.

From these reports, we can summarize that light irradiation inhibits PGE<sub>2</sub> and COX expression and reduces ROS by electron transition. But the relationship between ROS dissociation and PGE<sub>2</sub> release remains uncertain. We proposed the hypothesis that a specific wavelength of light irradiation induces ROS dissociation and results in inhibition of COX and PGE<sub>2</sub> release. To test this hypothesis, we studied the effects of 635 nm light irradiation compared with existing COX inhibitors indomethacin and ibuprofen.

## MATERIALS AND METHODS

### Primary Cell Culture and Chemicals

Human gingival fibroblasts (hGFs) were obtained from a healthy 34-year-old adult visiting Chonnam National University Hospital for gingivectomy. The gingival tissues were finely cut with scissors and cultured in an alpha minimum essential medium ( $\alpha$ -MEM) (GibcoBRL, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (Biomed Co., CA, USA) and 10% antibiotic-antimycotic solution (Welgene, Daegu, Korea) at 37°C in a 5% CO<sub>2</sub> humidified chamber. The medium was replaced with fresh medium, and the adherent hGF cells were allowed to reach about 70% confluence. Then the cells were detached using the trypsin-ethylenediamine tetraacetic acid (trypsin-EDTA: GibcoBRL) solution and then plated again (subcultured) in 6-well plates for each experiment.

In this experiment, hGF cells were used at passage 2 or 3 and treated with (1) 200  $\mu$ M arachidonic acid (AA) (Sigma, St. Louis, USA) alone in  $\alpha$ -MEM, (2) indomethacin (Sigma) after AA treatment in  $\alpha$ -MEM, (3) ibuprofen (Sigma) after AA treatment in  $\alpha$ -MEM, and (4) irradiation after AA treatment in  $\alpha$ -MEM. In the case of indomethacin or ibuprofen, 10 mM of indomethacin or ibuprofen was separately dissolved in 0.1% ethanol, diluted with  $\alpha$ -MEM medium according to the concentration indicated below, and treated to the cells, respectively.

Each treatment was performed for 1 hour, followed by a change to a fresh medium and incubation at 37°C in a 5% CO<sub>2</sub> humidified chamber. The control group did not receive any treatments.

### Light Source and Irradiation

The source of light for irradiation was a continuous-wave LED (U-JIN LED, Goyang-City, Korea) emitting at a wavelength of 635 nm, and the manufactured energy density

was 1 mW/cm<sup>2</sup> on the sample surface. The manufactured LED irradiation tool kit was built in a 5% CO<sub>2</sub> humidified chamber at 37°C (Biophoton, Gwangju, Korea).

### Enzyme-Linked Immunoassay for PGE<sub>2</sub>

The PGE<sub>2</sub> expression level, the main metabolite of COX, was used to determine the indomethacin and ibuprofen concentration in all subsequent experiments for this study. The amount of PGE<sub>2</sub> was measured in the supernatants using a commercially available enzyme immunoassay kit (R&D System, MN, USA) according to the manufacturer's protocol. AA-treated hGF cells were incubated with indomethacin (0.01, 0.1, 1, and 10 mM), ibuprofen (0.01, 0.1, 1, and 10 mM), or irradiation for 1 hour in  $\alpha$ -MEM. After 24 hours, absorbance for PGE<sub>2</sub> was measured at 586 nm by using a colorimetric microplate reader (Biotek, Winooski, USA).

### Total RNA Isolation and Real-Time Reverse Transcription-PCR (RT-PCR) for COX, ROS Scavenger (SOD, GPx, Catalase), and PLA<sub>2</sub>

At 6 hours after treatment, the total RNA was isolated using Trizol<sup>®</sup> Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. Quantitative real-time RT PCR analysis was performed using exicycler TM (Bioneer, Daejeon, Korea) with the following primer to amplify 100–150 bp amplicons: COX-1 forward 5'-TCCCACCATCTGATTAAC-3', reverse 5'-TGGTG-TGAGAACAAATGAGA-3', CO-X-2 forward 5'-CAGGCA-GATGAAATACCAGT-3', reverse 5'-CCATAGAGTGCTT-CCAACTC-3', SOD1 forward 5'-ACCTGCCCTACGACTA-CG-3', reverse 5'-GGTACTTCTCCT-CGGTGAC-3', SOD2 forward 5'-TCTCACTCTCAGGAGACCAT-3', reverse 5'-AATTACACCACAAGCCAAAC-3', GPx forward 5'-AGTC-GGTGTATGCCTTCTC-3', reverse 5'-CTCGTTCATCTGG-TGTAGT-3', Catalase forward 5'-CTTCTTGTTCAGGA-TGTGGT-3', reverse 5'-TGGTAATGTCATGTGTGACC-3', cPLA<sub>2</sub> forward 5'-CAGAAAAGTGG-GCTAAAATG-3', reverse 5'-GGCAATCTTCTCCATATCA-3', sPLA<sub>2</sub> forward 5'-AATTTCCCTCTCTCATACCC-3', reverse 5'-ATCTGCTGGATGTCTCATTC-3', iPLA<sub>2</sub> forward 5'-CCG-AGATCCATGAGTACAAT-3', reverse 5'-AA-GACATCCA-CACAGGTCAC-3', GAPDH forward 5'-CCAGTCAGCTT-CCCCTTCA-3' reverse 5'-GAACATCATCCCTGCATCCA-3'. Real-time RT PCR data were quantified by GAPDH for standard control.

### Western Blot Analysis

At 6 hours after treatment, the medium was removed and washed twice with phosphate-buffered saline (PBS, pH 7.4), then the cell lysate was prepared in 200  $\mu$ l of cold lysis buffer (1% NP-40, 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.02% sodium azide, 150  $\mu$ g/ml PMSF, 2  $\mu$ g/ml aprotinin, 20  $\mu$ g/ml leupeptin, and 1  $\mu$ g/ml pepstatin A). Approximately 30 mg of the cell lysate was separated in a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred onto a polyvinylidene difluoride membrane

(Amersham, NJ, USA). The membrane was blocked with blocking solution (5% skim milk in TBST (2.42 g/L Tris-HCl, 8 g/L NaCl, 0.1% Tween 20, pH 7.6)) for 0.5 hours and rinsed briefly in TBST. The membrane was incubated overnight at 4°C with the appropriate primary antibodies: rabbit polyclonal IgG anti-COX-1 (1:1,000) (sc-7950, Santa-Cruz, CA, USA), mouse monoclonal IgG anti-COX-2 (1:1,000) (sc-19999, Santa-Cruz Biotechnology). A mouse monoclonal IgG anti-beta-actin (sc-47778, Santa-Cruz Biotechnology) was used as a control.

After rinsing with TBST, the membrane was incubated for 1 hour with a horseradish peroxidase conjugated secondary antibody: anti-rabbit IgG-HRP (sc-2301, Santa-Cruz Biotechnology) (1:2,000) or goat anti-mouse IgG-HRP (sc-2031, Santa-Cruz Biotechnology).

Finally, the membrane was washed with TBST, and the immunoreactivity of proteins was detected with an enhanced chemiluminescence (ECL) detection kit (Amersham).

### Flow Cytometer and Laser Scanning Confocal Microscope Analysis for Detection of ROS Formation

The reactive oxygen species (ROS) were assayed by using 2',7'-dichlorodihydrofluorescein diacetate (H<sub>2</sub>DCF-DA; Sigma). DCF-DA enters cells passively, where it is enzymatically deacetylated by esterases to become the nonfluorescent 2,7-dihydrodichlorofluorescein (DCF-H). Meanwhile, the oxidizing molecules such as O<sub>2</sub><sup>-</sup>, convert DCF-H to the highly fluorescent DCF.

To visualize intracellular ROS dissociation, DCF fluorescence was checked at 10, 30, and 60 minutes, respectively. hGFs grown on cover slips were incubated with 10 μM of DCF-DA for 20 minutes. After the cells were washed with phosphate-buffered saline containing 10 nM of glucose, DCF fluorescence intensity was monitored using a confocal microscope (Carl Zeiss, Jena, Germany), set at the excitation and emission wavelengths of 488 and 525 nm, respectively. To measure the time-dependent intracellular ROS level, the cells were treated as above. After the treated cells were detached using a trypsin-EDTA solution, the ROS level was analyzed by flow cytometry (Beckman Coulter, CA, USA) using 485 nm of excitation and 530 nm of emission filters.

### Lipid Peroxidation Assay

At 24 hours after treatment, lipid peroxidation was measured by an MDA colorimetric assay kit (Oxford Biomedical Research, CA, USA). The colorimetric assay reagent was applied to the cells according to the manufacturer's instructions, and absorbance was measured at 586 nm using a colorimetric microplate reader (Bio Tek).

### Statistical Analysis

Data are expressed as means ± standard deviation. All experiments were repeated three to four times. For significance testing, Student's *t*-test was used ( $P < 0.05$ ).

## RESULTS

### PGE<sub>2</sub> Release and COX Expression

AA significantly increased PGE<sub>2</sub> release, while irradiation, indomethacin, and ibuprofen significantly inhibited PGE<sub>2</sub> release. Indomethacin and ibuprofen significantly inhibited PGE<sub>2</sub> release in a dose-dependent manner: 1 or 10 mM indomethacin and 10 mM ibuprofen reduced PGE<sub>2</sub> release more than LED irradiation, respectively. From these results, we chose the concentration of indomethacin and ibuprofen in all subsequent experiments: 1 mM indomethacin and ibuprofen, respectively (Fig. 1A,B).

To identify the inhibitory effects of irradiation, indomethacin, and ibuprofen on COX-1 and COX-2 expression, AA-stimulated hGF cells were examined by real-time RT-PCR (Fig. 1C,D) and Western blot analysis (Fig. 1E). COX-1 mRNA expression was slightly increased in the group treated with AA alone but was significantly inhibited in the irradiation group compared to indomethacin and ibuprofen (Fig. 1C). COX-2 mRNA expression was significantly increased in the group treated with AA alone but was significantly inhibited in the groups treated with 1 mM ibuprofen and irradiation (Fig. 1D).

COX protein expression was slightly increased in the group treated with AA alone and was decreased in other groups. In the case of the COX-2 protein similar to COX-2 mRNA expression, it was greatly increased in the group treated with AA alone and greatly decreased in the irradiation group (Fig. 1E).

### Intracellular ROS Level and mRNA Expression of ROS Scavenger by Irradiation Versus Indomethacin and Ibuprofen Treated Group

To elucidate the mechanism, the intracellular ROS levels were examined at 10, 30, and 60 minutes in the groups treated with irradiation, indomethacin, and ibuprofen (Fig. 2A,B). In the flow cytometric finding, the DCF fluorescence was decreased dramatically in the irradiation group compared to the 1 mM of indomethacin or ibuprofen group (Fig. 2A). In the microscopic finding, the DCF fluorescence obviously vanished in the irradiation group in a time-dependent manner, while there were no significant differences in the indomethacin and ibuprofen groups (Fig. 2B).

The effects of intracellular ROS scavenging were examined by real-time RT-PCR in AA-stimulated hGF cells treated with irradiation, indomethacin, and ibuprofen (Fig. 2C,D). The SOD1 levels were significantly increased in the group treated with AA alone, whereas irradiation significantly inhibited it (Fig. 2C). In the case of the SOD2 level, indomethacin and ibuprofen affected the dissociation (Fig. 2C). In the case of GPx mRNA expression, irradiation significantly inhibited expression (Fig. 2D). But in the case of catalase mRNA expression, expression was significantly inhibited in the groups treated with irradiation, indomethacin, and ibuprofen (Fig. 2D).

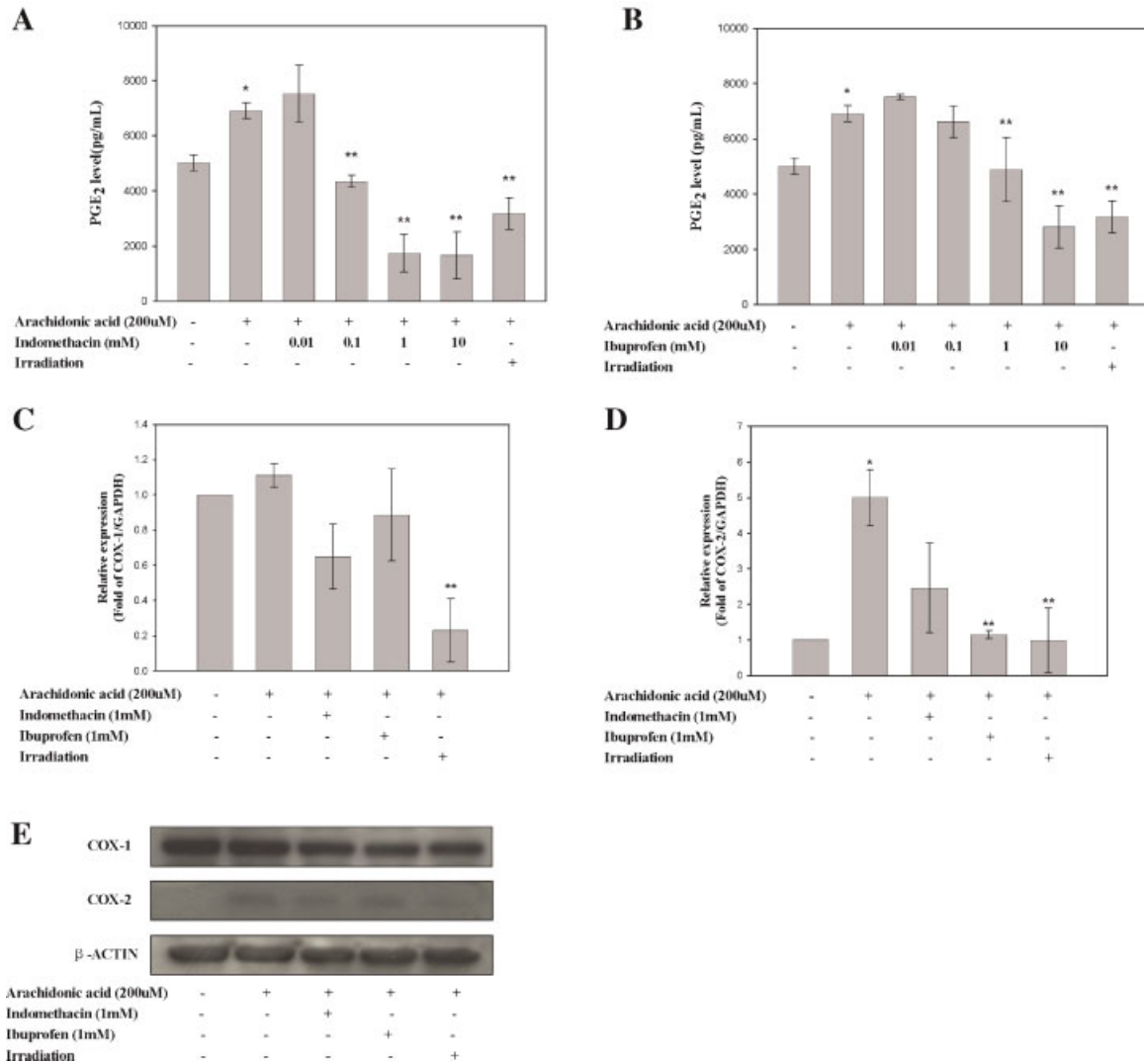


Fig. 1. Inhibition for PGE<sub>2</sub> release after treatment with different doses of indomethacin and ibuprofen was compared with that after irradiation treatment (A,B). mRNA (C,D) and protein (E) expression of COX-1 and COX-2 were tested at 6 hours after treatment with irradiation, indomethacin (1 mM), and ibuprofen (1 mM), respectively. Significant differences were seen at \* $P < 0.05$  compared to the control group and \*\* $P < 0.05$  compared to AA, respectively.

### mRNA Expression of PLA and Lipid Peroxidation Assay by Irradiation Versus Indomethacin and Ibuprofen

The changes of various phospholipase A<sub>2</sub> (PLA<sub>2</sub>) mRNA expression and lipid peroxidation results were shown (Fig. 3). Ca<sup>2+</sup> independent PLA<sub>2</sub> (iPLA<sub>2</sub>) was not a significant difference in all groups, but cPLA<sub>2</sub> and sPLA<sub>2</sub> mRNA expression was significantly inhibited by irradiation.

To investigate the lipid peroxidation level by irradiation, indomethacin, or ibuprofen, a malondialdehyde (MDA) colorimetric assay was performed. The MDA level was decreased in all the groups treated with irradiation,

indomethacin, and ibuprofen compared to the group treated with AA alone, but there was no significant difference (Fig. 3C).

### DISCUSSION

The present study investigated the physiological mechanism of the anti-inflammatory effects of 635 nm light irradiation on AA-treated human gingival fibroblasts (hGFs) and compared them with existing COX inhibitors: indomethacin and ibuprofen. In the results of the present study, 635 nm irradiation and existing COX inhibitors inhibited the expression of COX and PGE<sub>2</sub>. Unlike indomethacin or ibuprofen, 635 nm irradiation decreased the ROS level and mRNA expression of cPLA<sub>2</sub> and sPLA<sub>2</sub>.

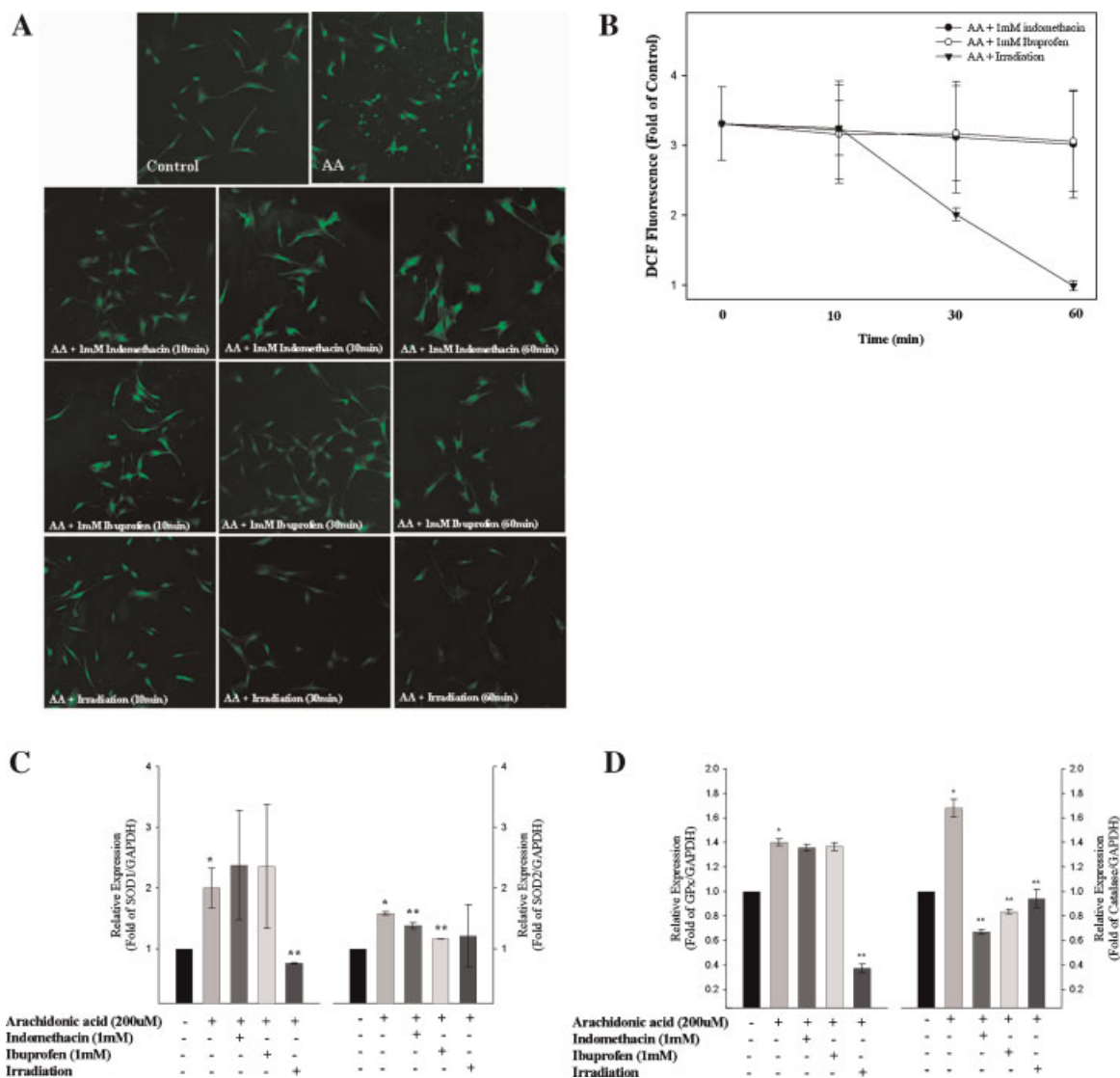


Fig. 2. The confocal microscopic analysis of the ROS level in groups treated with 1 mM indomethacin and ibuprofen was compared with that of the group treated with irradiation in a time-dependent manner (A). Treatment with 1 mM of indomethacin, ibuprofen, and irradiation was performed for the indicated time (10, 30, and 60 minutes). The ROS level was

measured by flow cytometer (B). SOD1, SOD2 (C), GPx, and catalase (D) mRNA expression of irradiation were compared with those of the groups treated with indomethacin (1 mM) and ibuprofen (1 mM). Significant differences were seen at  $*P < 0.05$  compared to the control group and  $**P < 0.05$  compared to AA, respectively.

The 635 nm light irradiation can play a role in anti-inflammation similar to indomethacin and ibuprofen, which are known to be COX inhibitors [1,22]. Constitutively expressed COX-1 is present in most tissues, where it synthesizes PGE<sub>2</sub> continuously to maintain physiological functions, while COX-2 is induced by pro-inflammatory stimuli, cytokines, and mitogens and affects the synthesis of a large quantity of PGE<sub>2</sub> [22]. In the present study, mRNA and protein expression of COX-1 and -2 was significantly inhibited by irradiation as compared to existing COX inhibitors. Ibuprofen led to a more effective

decrease in COX-2 mRNA expression than indomethacin. From these results, irradiation down-regulated the COX enzymes and PGE<sub>2</sub> release resulted in anti-inflammatory function. In general, ibuprofen is known to be a COX-2 selective inhibitor developed against the side effects of COX-1 inhibitors [6]. Considering the selectivity as a COX inhibitor, we suggest that irradiation has a function as a COX inhibitor. Unlike other COX inhibitors, irradiation has not been assessed for side effects yet. The side effects of COX-1 inhibitors are associated with gastrointestinal injury, whereas irradiation was directly applied to the

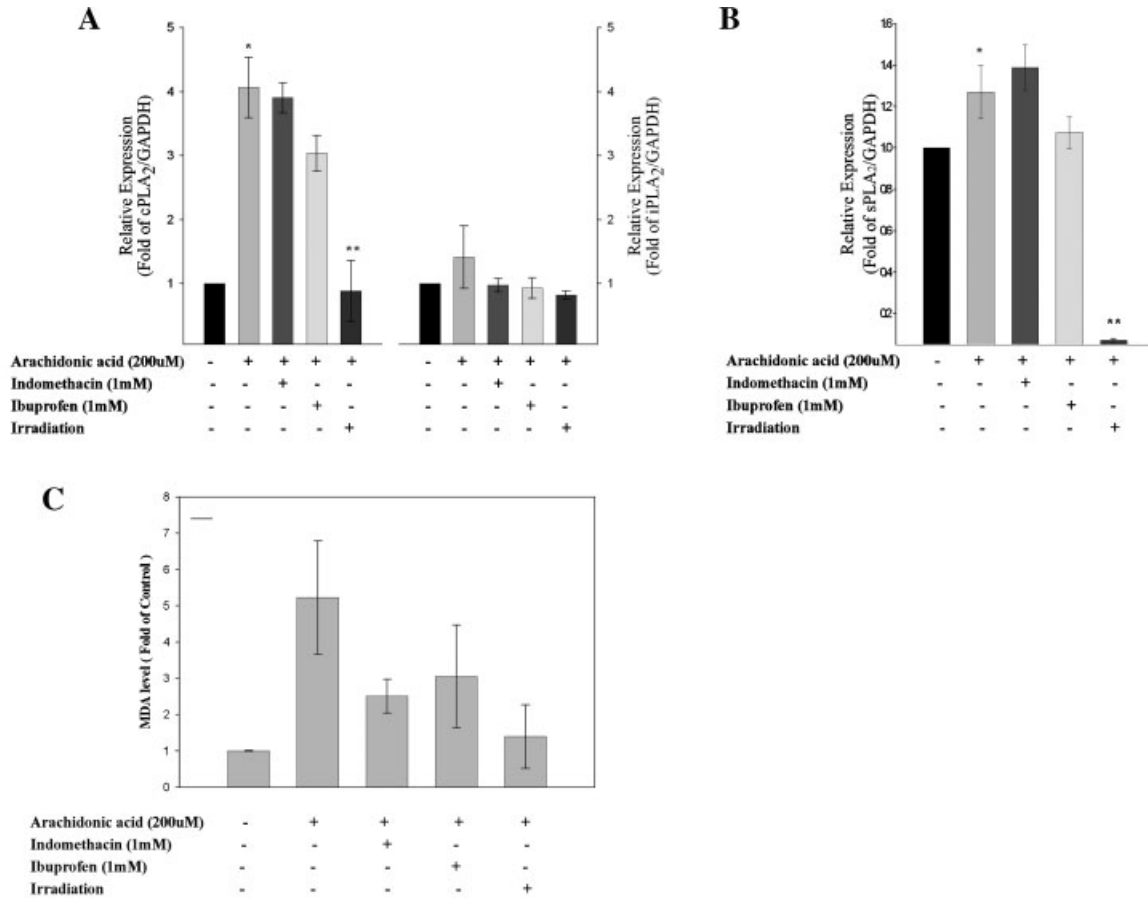


Fig. 3. cPLA<sub>2</sub>, iPLA<sub>2</sub> (A), and sPLA<sub>2</sub> (B) mRNA expression of irradiation were compared with those of the groups treated with indomethacin (1 mM) and ibuprofen (1 mM) after 6 hours. The lipid peroxidation assay by MDA determination of irradiation was compared with those of the groups treated with indomethacin (1 mM) and ibuprofen (1 mM) (C). Significant differences were seen at \* $P < 0.05$  compared to the control group and \*\* $P < 0.05$  compared to AA, respectively.

wound, which has not shown possible side effects yet. Further study is needed to explore in detail the side effects of irradiation.

How can 635 nm light irradiation down-regulate the COX enzymes and decrease PGE<sub>2</sub> release? To answer this question, the intracellular reactive oxygen species (ROS) level was evaluated. It was known that COX-2 was crucial to the ROS response [16]. Particularly, the study of induced oxidative stress has been explained by the effects of arachidonic acid. Recent reports have shown that ROS induced by arachidonic acid leads to cell oxidative injury, such as DNA damage and lipid peroxidation [23]. ROS are also mediated by the enzyme related to the reaction of the metabolism of the unsaturated fatty acid. In the present study, the ROS level was decreased as time passed in the irradiation group, which meant that irradiation functions as a ROS scavenger even though its mechanism is not clear. Several studies coincided to some extent in that superoxide

anion can be dissociated by UV or visible light irradiation. This photo-dissociation can occur due to low-energy photons such as sunlight [20]. That is, 635 nm irradiation rapidly dissociated the intracellular ROS in AA-treated hGFs. But indomethacin and ibuprofen could not decrease the ROS level, which means that they are not associated with ROS dissociation. This point is the different way of COX inhibition between irradiation and existing COX inhibitors in AA-treated hGFs.

The intracellular ROS level may be defined as an imbalance between cellular release and antioxidant defense mechanisms such as superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD2), glutathione peroxidase (GPx), and catalase (CAT) [24,25]. In the present study, mRNA expression of SOD1, GPx, and catalase was down-regulated by irradiation, while SOD2 and catalase were down-regulated by existing COX inhibitors. From these results, irradiation can act as a ROS scavenger and

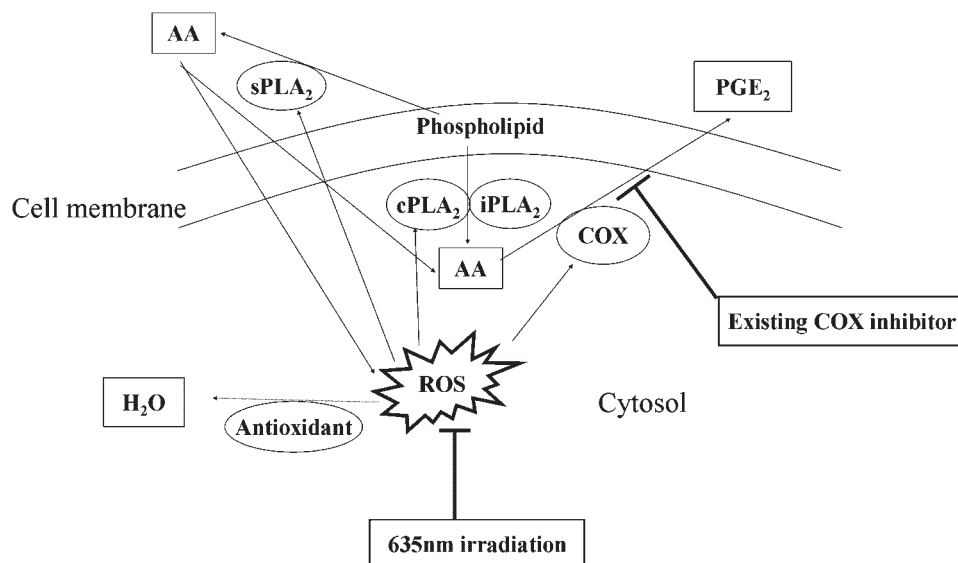


Fig. 4. A diagram shows the putative pathways of ROS and PGE<sub>2</sub> release along with the possible points of 635 nm irradiation and COX inhibitor involvement. Intracellular ROS is increased by AA treatment, and scavenged by intracellular antioxidant such as superoxide dismutase (SOD) and catalase. But over-production of ROS incapable of being dissociated by the intracellular anti-oxidant may induce the

mRNA expression of cPLA<sub>2</sub>, sPLA<sub>2</sub>, COX-2, and hence promote PGE<sub>2</sub> release. The existing COX inhibitor is known to block the activity of COX-1 or COX-2, but has little relation with ROS dissociation. In the present study, 635 nm irradiation shows that it can directly dissociate the intracellular ROS. This leads to the inhibition of cPLA<sub>2</sub>, sPLA<sub>2</sub> and COX-2 expression, finally resulting in a decreased release of PGE<sub>2</sub>.

balance the intracellular ROS level. As compared to indomethacin and ibuprofen, irradiation affected the antioxidant, which coincided with the ROS level results.

Some reports have shown that a synergistic interaction exists between PLA<sub>2</sub> and ROS in which membranes exposed to ROS are peroxidized and become more susceptible to PLA<sub>2</sub> action [26]. In the present study, mRNA expression of cPLA<sub>2</sub> and sPLA<sub>2</sub> was decreased by irradiation. It can be assumed that irradiation down-regulates cPLA<sub>2</sub> and sPLA<sub>2</sub> through a reduction of ROS, eventually resulting in PGE<sub>2</sub> inhibition. In addition, the MDA assay showed that lipid peroxidation was more decreased by irradiation than by indomethacin and ibuprofen.

Considered the mechanism of anti-inflammatory effects by 635 nm irradiation based on the present study, ROS was scavenged by the intracellular anti-oxidation pathway of SOD, GPx, and catalase. Irradiation can also dissociate the ROS directly, which inhibits cPLA<sub>2</sub>, sPLA<sub>2</sub>, and COX expression, resulting in inhibition of PGE<sub>2</sub> release (Fig. 4).

Finally, 635 nm LED irradiation, indomethacin, and ibuprofen can inhibit COX enzyme and PGE<sub>2</sub> release, which results in anti-inflammation. However, the biological mechanism of irradiation is somewhat different from that of indomethacin and ibuprofen.

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