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# 구강암에서 5-Aminolevulinic Acid-Hexenyl Ester의 광역학치료 효과

조선대학교 대학원

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이 논문을 치의학 석사학위신청 논문으로 제출함

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# ABSTRACT

## Photodynamic Efficacy with a Novel Hexenyl Ester of 5-Aminolevulinic Acid in Oral Cancer Cells

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5-Aminolaevulinic acid (ALA) and its derivatives act as precursors to the photosensitizer protoporphyrin IX (PpIX). It can be used clinically as a photosensitizer for photodetection or photodynamic therapy (PDT) of cancer. It has been known that ALA enhances cellular accumulation of PpIX and is readily hydrolysed to get into the heme biosynthetic pathway, which produces PpIX on cells. In the present study, we compared the amount of PpIX synthesis of ALA and its hexenyl ester of ALA (ALA-hx) in human oral squamous cell carcinoma (OSCC) cell line. We also compared the effectiveness of reactive oxygen species (ROS) and the modification of cell death induced by PDT. PpIX synthesis of ALA-hx was much higher than that of ALA. In addition, ALA-hx-induced PDT generated ROS and led to inhibition of cell growth. PDT with ALA-hx induced activation of apoptosis related proteins such as caspase-3/7/PARP. Subsequently, apoptotic pattern was clearly demonstrated by flow cytometry. These results suggested that ALA-hx-PDT induced apoptotic cell death via a mitochondria-mediated pathway. These findings provide the improvement efficacy of ALA-hx mediated PDT as an alternative candidate for human oral cancer therapy.

# 국문초록

## 구강암 세포주에서 광감작제인 5-ALA-hexenyl의 광역학치료 효과

광역학 치료는 광민감제에 특정 광원을 조사하여 세포내에서 활성산소종(Reactive oxygen species, ROS)의 증가를 통한 암세포의 사멸을 유도하는 방법이다. 5-Aminolaevulinic acid (ALA)는 heme의 생합성 과정의 대사 전구물질로서 조직 내에서 heme의 전구물질인 광민감제 protoporphyrin IX (PpIX)으로 전환된다. 외인성 ALA를 적용하면 내인성 광민감제인 PpIX을 형성하게 되고, PpIX이 광민감제로 활성화되면 형광을 나타내고 세포 독성을 나타내는 특성이 있다. 따라서 이 연구에서는 사람의 구강암 세포주인 YD-10B 세포를 통해 기존의 ALA와 이의 유도체인 ALA hexenyl ester (ALA-hx)의 PpIX 형성을 비교하고, 광역학치료 후 활성 산소종의 형성능과 세포사의 분자기전을 확인하였다. 연구결과 ALA 처리 그룹보다 ALA-hx 처리 이후 PpIX의 형성이 더 빠르게 증가하며, ALA-hx 적용 후 미토콘드리아로 위치함을 관찰할 수 있었다. 세포증식율 또한 ALA-hx에 의한 광역학치료 후 ALA 보다 세포증식 억제에 더욱 효과적임을 확인할 수 있었다. 또한 ALA-hx의 광역학치료 후 활성산소종의 증가와 apoptosis의 유도를 확인하였고, caspase-3/7/PARP의 활성화를 관찰하였다. 결과적으로 ALA-hx는 기존의 ALA 보다 구강암 세포의 성장 억제에 더욱 효과적이며, 활성 산소종에 따른 미토콘드리아 의존적 경로를 통해 apoptosis가 유도되었음을 확인할 수 있었다.

# I . Introduction

Oral squamous cell carcinoma (OSCC) ranks among the top ten most commonly occurring cancers worldwide. Despite recent improvements in surgery and radiation therapy, as well as advances in chemotherapy, the 5-year survival rate for oral cancer patients has remained at 50% over the past five decades.[1,2] Several clinical applications of surgery, radiation, and chemotherapy are standard treatments for early oral squamous cell carcinoma, which result in good tumor control. However, there are many severe side effects and/or toxicity in patients.[3] Therefore, alternative therapeutic strategy has been demanded.

Photodynamic therapy (PDT) has been developed as an alternative therapy for cancer treatment. PDT is based on the interaction of a photosensitizer and visible light with a suitable wavelength to cause the destruction of cancer cells by the formation of cytotoxic reactive oxygen species (ROS).[4] PDT with 5-aminolaevulinic acid (ALA) is accepted world wide for treatment of cancerous and non-cancerous diseases. ALA and its derivatives act as precursors to the photosensitizer protoporphyrin IX (PpIX), which is a potent photosensitizer.[5,6] ALA itself is not a photosensitizer and serves as the biological precursor in the heme biosynthesis pathway.[4-7] If extra exogenous ALA is introduced into cells, endogenous PpIX formation will be enhanced and accumulated in cells.[8] Upon light exposure, PpIX is converted into its excited triplet state, enabling energy transfer to ground state molecular oxygen, which is then converted to reactive singlet oxygen. The latter is capable of inducing cell death such as necrosis or apoptosis.[9] As a doubly charged molecule, ALA does not penetrate easily across cell membranes.[10] To overcome the limit of uptake and distribution of ALA, the drug has been converted into its esters to increase its lipophilic nature.[11-13] Several ALA esters has been synthesized and investigated as promising precursors to PpIX in vitro and in vivo as well.[13]

The esterified ALA derivatives significantly increase PpIX levels over ALA in various cell lines.[8-13] As the importance of PDT has increased, a new efficient synthetic methodology for a variety of esters of ALA is necessary. Although synthesis and increased bioavailability of ALA-esters from saturated hydrocarbon alcohols have been reported, esters of ALA with unsaturated alcohols have not been reported in the literature.[14] ALA-hexenyl ester (ALA-hx), as a unsaturated alkyl ester, increased the PpIX accumulation in cell compared to that by ALA in a broad range of concentrations.[14,15] This novel ALA-hx-mediated PDT significantly blocked the tumor cell proliferation and induced cell death in vitro.[15]

In this study, we compared the amount of PpIX as well as ROS production between ALA and ALA-hx in human OSCC cell line in vitro. We also investigated the modification of cell death induced by ALA-hx-mediated PDT.

# Ⅱ. Materials and Methods

### 1) Cell culture and reagents

An oral squamous cell carcinomas cell line, YD-10B cells were purchased from Korea Cell Line Bank (KCLB, Seoul, Korea). The cells were maintained in RPMI medium containing 5% fetal bovine serum and 100 U/ml penicillin-streptomycin (Invitrogen, CA, USA), and subsequently incubated at 37°C in an atmosphere containing 5% $CO_2$. ALA was purchased from Sigma (St Louis, MO, USA). The ALA hexenyl ester (C11H19NO3·HCl, ALA-hx) were made as previously described.[14] ALA and ALA-hx was dissolved in serum-free RPMI medium immediately before use.

### 2) Measurement of PpIX

The cells ($2\times10^4$ cells/well) were cultured in a 4-chamber slide. To examine the generation of PpIX, the cells were incubated in serum-free media with 300 μM ALA and ALA-hx for 4 h in darkness. The fluorescence of PpIX in the cells was observed with conventional fluorescent microscope (Olympus IX 71, Olympus America Inc., Melville, NY) and Olympus MicroImage software.

The quantity of PpIX is analyzed immediately by Thermo Scientific Varioskan® Flash spectral scanning multimode reader (Varioskan, Thermo Electron Co, Waltham. MA, USA) with SkanIt® Software (Wavelength : Excitation 408 nm, Emission 573-800 nm).

### 3) RT - PCR analysis for Coproporphyrinogen oxidase (CPO)

Total RNA was harvested from cells with Trizol Regent (Invitrogen, Carlsbad, CA) exactly as described by the manufacturer.[16] One micrograms of total RNA was reverse transcribed into cDNA and PCR was performed using the Superscript™ One-step RT-PCR with platinum® Taq kit (Invitrogen, Carlsbad, CA). To detect semiquantitative changes in the level of mRNAs for

CPO in cells, the following primer sets were used. CPO: sense, 5'-CTCCTACTATCCATTTCAACTACAG-3' CPO: antisense, 5'-CACGATGG GCTATAAAGAAGTAATC-3'. GAPDH primer sense, 5'-CCAAGGTCATCCA TGACAACTTTG-3', antisense, 5'-GTCATACCAGGAAATGAGCTTGACA-3' was used as internal control. For PCR amplification, cycle conditions were as follows: 50℃ 30 min, 94℃ 30 s, 94℃ 30 s, 55℃ 30 s, 72℃ 30 s × 29 cycles, and 72℃ 10 min. One microliter of PCR products were then electrophoresed on a 5% acrylamide gel, visualized, and photographed using a gel documentation system.

### 4) Localization of PpIX

To examine the localization of PpIX, the cells ($3\times10^4$ cells/well) were incubated with 1 μM ALA-hx in serum-free media for 4 h. To stain mitochondria, Mito-Tracker Red (Molecular Probes, Inc, Eugene, OR, USA) was added to a final concentration of 20 nM. PpIX localization in the cells was imaged with excitation of 488 nm and emission of 560 to 590 nm using a Laser confocal scanning microscope TCS SP5 TANDEM (Leica-Microscope systems, Leica, Germany).[7,12] The images were displayed with different interference contrast images of green and red false color.

### 5) Photodynamic treatment

YD-10B cells were washed with PBS and incubated in serum-free culture medium containing each photosensitizer of ALA and ALA-hx. Four hours after ALA and ALA-hx administration, the cells were exposed with light doses of 5 J /$cm^2$ of a red light-emitting diode (LED) light with 613-645 nm wavelength, with peak 635 nm (Philips Luxeon Lumileds, San Jose, CA, USA.) at 35 mW /$cm^2$ as measured with a Delta Ohm DO 9721 quantum photo-radiometer and thermometer data logger (Model DO9721, Padua, Italy).[15]

### 6) MTT assay

The cytotoxic effect of PDT on the survival of ALA- and ALA-hx-treated cells was examined in time- and dose-dependent manner using MTT assay (3-(4,5-dimethylthiazol-2-yl)-3-5-diphenyltetrazolium bromide). $1\times10^5$ cells/well were cultured in 12 well plates. After 24 h, the cells were washed with PBS and added with each photosentizer to the serum-free media. The cells were incubated with photosensitizer for 4 h, followed by irradiation. Immediately after PDT, the cells were given with fresh media. At 12, 24, and 36 h after PDT, the cell viability was assessed using a MTT reagent . In addition, the cells were incubated with each concentration of ALA-hx. Each well was washed twice with PBS, and 0.5 mL of cell culture media and 50 $\mu$l of 3-(4,5-dimethylthiazol-2-yl)-3-5-diphenyltetrazolium bromide solution (5 mg/mL in PBS) were added. After 3 h of incubation, media was removed and 250 $\mu$l of acid-isopropanol (0.04 mol/L HCL in isopropanol) was added. The absorbance of the reaction solution was measured at 595 nm of Microplate Autoreader ELISA (Bio-Tek Instruments Inc., Winooski, VT) and all experiments were performed in triplicate.

### 7) Measurement of intracellular ROS

After PDT, the cells were washed twice with PBS and incubated in medium containing 10 $\mu$M 2´7´-dichlorofluorescein diacetate, $H_2$DCFDA (Molecular Proves$^{TM}$, Eugene, Oregon) at 37°C in an atmosphere containing 5% $CO_2$ for 30 min. Fluorescence was analyzed immediately by Thermo Scientific Varioskan® Flash spectral scanning multimode reader (Varioskan, Thermo Electron Co, Waltham. MA, USA) with SkanIt® Software (Wavelength: Excitation 450-495, Emission 517-527).

### 8) Flow cytometry

At 24 h after PDT, the cells were stained with Alexa Fluor 488 Annexin V and propidium iodide (Vybrant Apoptosis Assay kit #3, Molecular Probes,

Eugene, Oregon, USA), according to the manufacturer's protocol. After incubated for 15 min in the darkness at room temperature, the $1\times10^4$ cells per sample were analyzed immediately using Cell Llab QuantaTM SC flow cytometer (Beckman Coulter Inc, Miami) and software.

### 9) Caspase-3 activity

Activity of caspase-3 in the cells with PDT was measured using ApoAlert Caspase Colorimetric Assay kit according to the manufacturer's instructions (Clontech Laboratories, Mountain View, CA). The absorbance of the solution was measured at 405 nm using Microplate Autoreader ELISA (Bio-Tek Instruments Inc., Winooski, VT) and all experiments were performed in triplicate.

### 10) Western Blot

At 24 h after PDT, the cells were lysed in 1 × reporter lysis buffer (Promega, Madison, WI). Then, protein concentrations were measured at 595 nm using Microplate Autoreader ELISA (Bio-Tek Instruments Inc., Winooski, VT). The total protein was resolved by 12% SDS-PAGE and transferred onto PVDF membranes. After blocking in TBS-T (20 mM/L Tris, 137 mM/L NaCl, 1 g/L tween 20, pH 7.6) with 5% skim milk for 4 h at room temperature, the membranes were incubated with primary antibodies against caspase-3/7/9, clraved PARP (diluted 1:1000; Cell Signaling Technology, Beverly, MA), actin, and Bcl-2 (diluted 1:1000; Santa Cruz Biotechnology, Inc. USA) for 2 h. The membranes were then washed three times with TBS-T and incubated with secondary antibodies for 1 h at room temperature. Finally, the membranes were visualized with West-ZOL® (plus) (iNtRON Biotechnology Inc., Korea) detection reagent using chemiluminescence system of LAS-1000 Image Reader of Luminescence Image Analyzer (FUJIFILM Life Science, Tokyo, Japan).

### 11) Statistical analysis

The differences in mean values among different groups were tested, and the values were expressed as mean ± SD. All of the statistical calculations were carried out using Microsoft Excel.

# Ⅲ. Results

### 1) Comparison of PpIX synthesis and CPO mRNA expression by ALA and ALA-hx

After treated with each photosensitizer, productions of PpIX were measured in time-dependent manner. PpIX synthesis was induced more rapidly by ALA-hx than ALA after 1, 2, 3, and 4 h each following incubation with the same concentration (Fig. 1A). Also, cells treated with both compounds for 2 and 4 h. PpIX red fluorescence was increased by ALA-hx more than ALA (Fig. 1B). We next investigated CPO mRNA level, which may be rate-limiting and in the regulation of PpIX production.[16] Expression of CPO gene was higher in cells treated with ALA-hx than in cells treated with ALA (Fig. 1C, upper panel). Furthermore, CPO mRNA expression was quantified using the MultiGauge software. It showed a increase pattern of expression of CPO in ALA-hx compared with the ALA treatment group (Fig. 1C, lower panel).

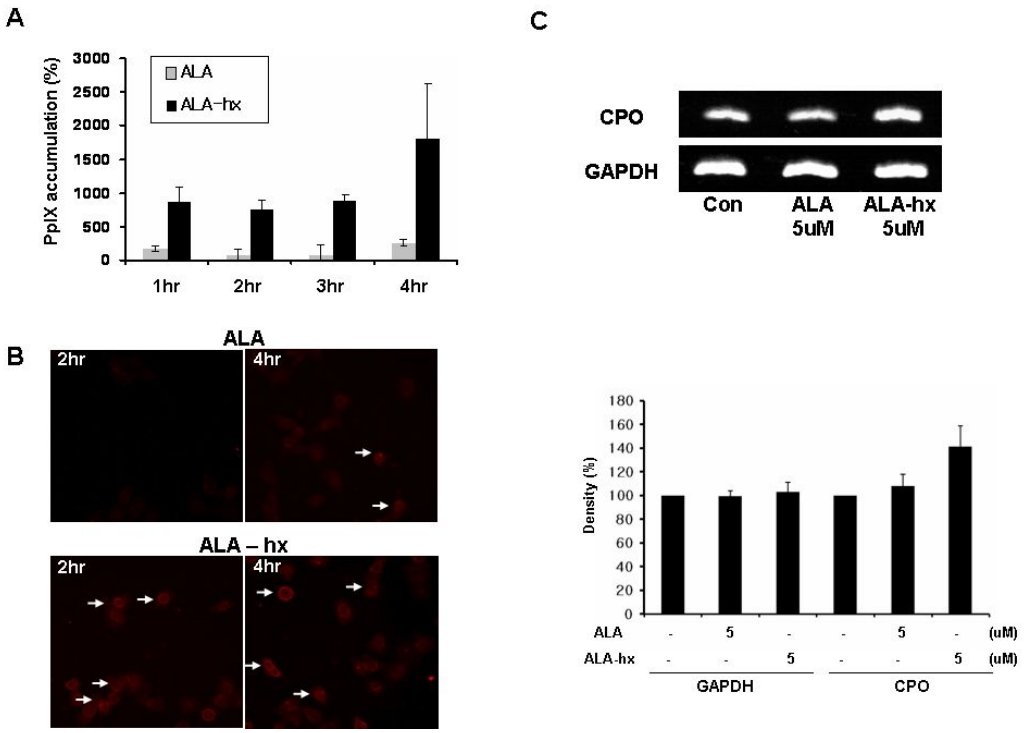


**Fig.1. PpIX accumulation and CPO mRNA expression after treatment of ALA and ALA-hx.** (A) Cells were incubated with 5 $\mu$M ALA and ALA-hx in serum-free medium for 1, 2, 3, and 4 h. (B) The accumulation of PpIX showed a linear increase up to 4 h (arrow). (C) Expression of CPO mRNA was increased by ALA-hx treatment compared with ALA.

### 2) Intracellular localization of PpIX by ALA-hx

Cellular photodamages associated with PDT have been ascribed to cytoplasmic membrane, mitochondria, lysosome, endoplasmic reticulum (ER), and Golgi depending on the photosensitizer.[7] The different subcellular localization of photosensitizer might induce different cellular effects by either rescue response or undergoing cell death. Intracellular accumulation of PpIX was observed to be mitochondrial potential fluorescence after treatment of 1 $\mu$M ALA-hx for 4 h. As shown in Figure 2, accumulation of PpIX following ALA-hx treatment

was mainly localized at mitochondria. These results indicate that mitochondrial photodamages could be induced after light irradiation.

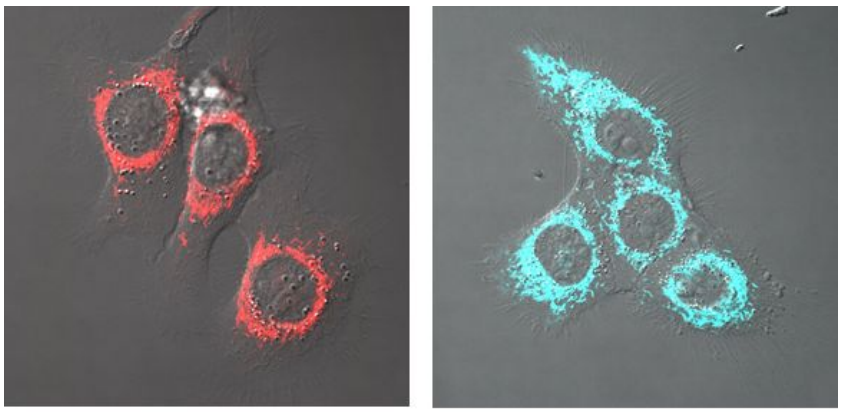


**Fig. 2. Mitochondria-potential localization of PpIX following ALA-hx treatment.** After 4 h incubation with 1 $\mu$M ALA-hx and 20 nM Mitotracker, intracellular PpIX mainly localized to the mitochondria. PpIX accumulation was mainly localized in the mitochondria.

### 3) ALA-hx-PDT reduces cell viability

The cytotoxic effects of ALA- and ALA-hx-mediated PDT in YD-10B cells were measured in time- and dose-dependent manner using 3-(4,5-dimethylthiazol-2-yl)-3-5-diphenyltetrazolium bromide. After treated with each photosensitizer for 4 h, cells were then irradiated. As shown in Fig. 3A, cell survival decreased depending on ALA-hx concentration compared with the control. Especially, cells induced an 88.5 % decrease at 24 h following PDT with ALA-hx compared with the control group. Moreover, the cells were measured to 0, 12, 24 h after PDT. At each time point, cell viability decreased time-dependently after treatment of ALA-hx PDT. The cells induced a 52.5% decrease at 24 h following ALA-hx compared with ALA (Fig. 3B). The group with the treatment of LED or photosensitizer alone did not show any change.

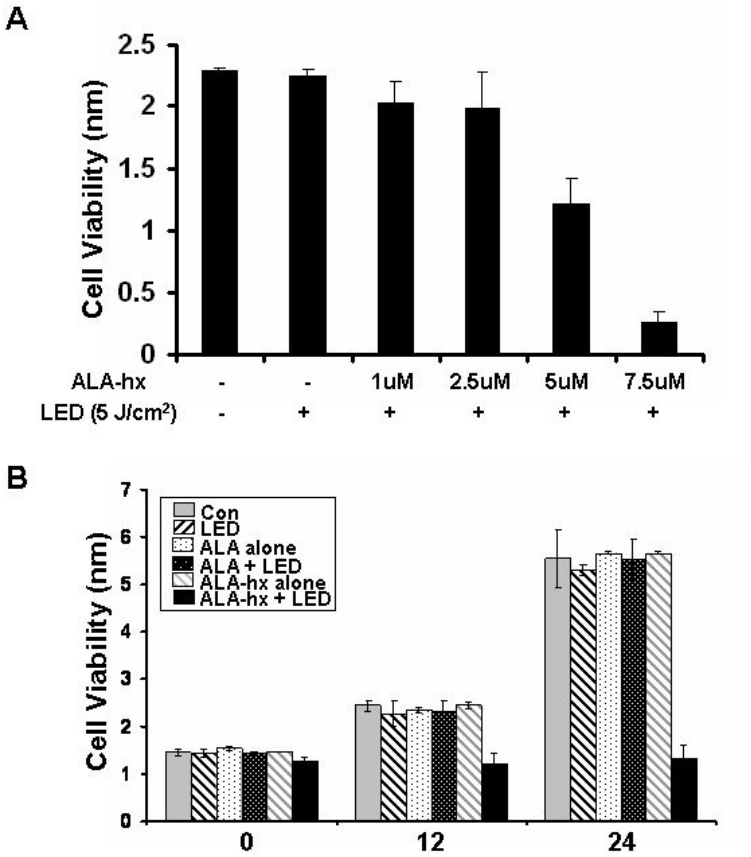


**Fig. 3. MTT assay.** (A) The cells were incubated with various concentration of ALA-hx for 24 h and then irradiated with 5 J/cm$^2$ of LED. (B) Cytotoxicity after PDT with treatment 5 $\mu$M of ALA and ALA-hx followed by time-dependent manner.

### 4) Generation of ROS

Production of ROS by PDT can lead to damage of the cellular constituents and subsequent cell death.[4,17] It is believed that ROS plays a direct role in damaging cells subjected to PDT.[18] To evaluate the generation of ROS, we used a green filter of inverted fluorescence microscopy. After PDT, the cells were examined for generation of ROS following PDT. ROS generation was

induced immediately after PDT and increased in a time-dependent manner compared with the control group (Fig. 4A). In addition, we also measured ROS production using the scanning multimode reader and Software. It was rapidly generated after ALA-hx-induced PDT and showed a significant increase pattern in time-dependent manner (Fig 4B).

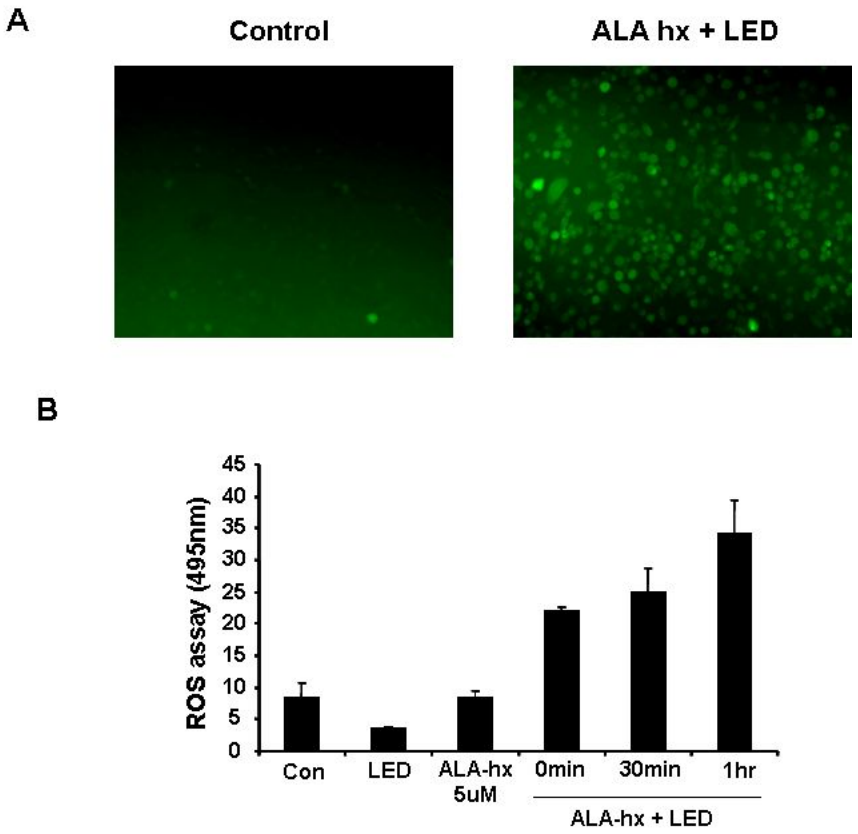


**Fig. 4. Generation of ROS.** Cells were incubated with 5 $\mu$M ALA-hx followed by exposure to 5 J/cm$^2$ of laser light. Quantitative amount of ROS production was measured using the scanning multimode reader and Software. (A, B) ROS generation was induced immediately after ALA-hx-PDT and increased in a time-dependent manner.

### 5) ALA-hx-PDT induces apoptosis and activates caspase-3 activity

We examined whether the growth-inhibitory effect of ALA-hx-PDT on the tumor cells was caused by apoptosis using flow cytometry. As shown in Fig. 5A, the number of apoptotic cells increased to the rate of 29.08 % at 24 h after ALA-hx-PDT. In addition, caspase-3 activity increased in ALA-hx PDT-treated cells depending on time after irradiation. The activation of effector enzyme such as cleaved-caspase-3/-7 and cleaved-PARP in apoptosis signaling pathway increased after 24 h by ALA-hx PDT (Fig 5C). In contrast, the expression level of Bcl-2, an anti-apoptotic protein, decreased. These data suggest that ALA-hx PDT induced the apoptosis through the caspase-3, caspase-7, PARP activation.

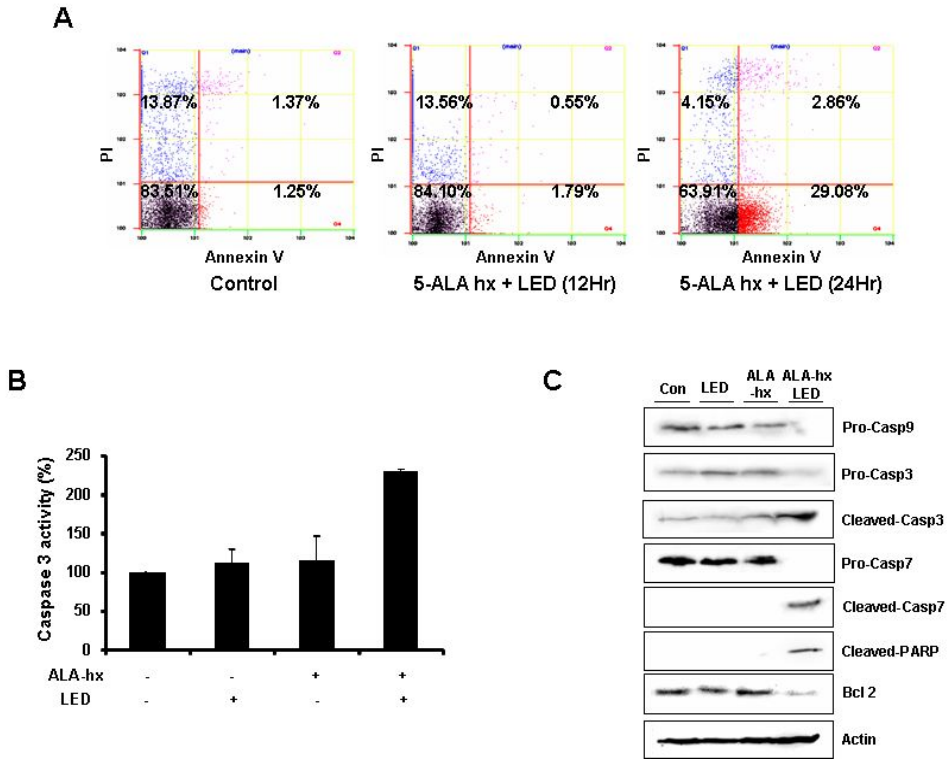


**Fig. 5. Flow cytomety and caspase-3 activity, and western blot analysis.** (A) Cells were incubated with 5 $\mu$M of ALA and ALA-hx for 4 h followed by exposure to 5 J/cm$^2$ of LED. Quantitative analysis of the apoptotic cells using annexin V and propidium iodide (PI) in exponentially growing control cells and PDT treated cells. (B, C) Caspase-3 activity and Western blot analysis of apoptosis-related protein following PDT. (B) Caspase-3 activity after PDT with 5 $\mu$M of ALA-hx. The legend shows time intervals after PDT. (C) PDT with ALA-hx induced activates apoptosis related proteins such as caspase-3/-7 and PARP. In contrast, cell survival factor Bcl-2 was reduced.

# Ⅳ. Discussion

ALA and its derivatives act as metabolic precursor to the PpIX for heme biosynthetic pathway and can be administered topically and systemically for PDT.[4-16,19] Although ALA has several advantages over other photosensitizers, its high hydrophilicity gives rise to relatively poor transport across cell membranes.[11-13,20] In order to increase the effectiveness of ALA, lipophilic prodrug, which upon entering a cell is hydrolysed by esterases, is a well known concept in pharmacology.[20] The use of the esterified ALA derivatives has been shown to induce of PpIX production compared to ALA.[5,8-15,19-20] Among ALA ester group, ALA-hx increased the PpIX accumulation in A431 cell compared to that by ALA and ALA-me a broad range of concentrations.[14,15] At lower concentrations, ALA-hx induced more significantly PpIX synthesis than that by ALA and ALA-me. These results suggest that unsaturated alkyl ester ALA-hx has slightly enhanced cellular uptake and is readily hydrolyzed to get into the heme biosynthetic pathway to produce photosensitizer PpIX on cells.[14,15]

In the present study, we examined the ability to induce PpIX synthesis and its relationship to the PDT response in human OSCC cell lines. PpIX synthesis was produced more rapidly ALA-hx than in ALA after each time-point following incubation with the same concentration. Amount of PpIX synthesis induced much more in ALA-hx than in ALA under the same concentration (Fig. 1A, B). Additionally, ALA-hx PDT induced the enhanced CPO mRNA expression, as a major late limiting enzyme for PpIX accumulation, in cells (Fig. 1C, D). PpIX accumulation was mainly localized in the mitochondria (Fig. 2). These results indicate that mitochondrial photodamages could be induced after light irradiation. In addition, when compared with ALA, the higher PpIX accumulation by ALA-hx is, the more sensitive cell responses to PDT become (Fig. 3A, B). Furthermore, cell

viability was decreased dose-dependently after treatment of ALA-hx PDT (Fig. 3B). We then tested whether the ROS were affected in PDT-treated cells. ROS, together with singlet oxygen produced via Type 2 pathway, are oxidizing agents that can directly react with many biological molecules.[17-18,21] To evaluate the generation of ROS, we observed using fluorescence microscopy and scanning multimode reader (Fig. 4A, B). After the PDT, the cells were measured for generation of ROS following by 5 J/ $cm^2$ of laser light and 5 $\mu$M of ALA-hx. ROS was generated higher in PDT-treated cell and showed a significant increase pattern of time-dependent manner (Fig. 4A, B).

PDT can lead to cell death such as necrosis or apoptosis.[9] It is increasingly appreciated that apoptosis is involved in PDT-mediated tumor cell death in vitro and tumor ablation in vivo.[18,21] Mitochondria have been demonstrated to perform a crucial function in the events resulting in caspase activation in many cell types undergoing apoptosis. In particular, the release of cytochrome c from mitochondria promotes procaspase-9 activation.[22] The activated caspase-9 cleaves the downstream effector caspases, including caspase-3, -6, and -7, which lead to the hallmarks of apoptosis.[23] To examine whether the growth-inhibitory effect of ALA-hx PDT is caused by apoptosis or necrosis, flow cytometry was performed 12 h and 24 h after ALA-hx PDT. The number of apoptotic cells increased with the rate of 29.08% at 24 h after ALA-hx PDT. In addition, caspase-3 activity was measured to examine whether ALA-hx-PDT induced apoptosis was caused by the pathway through caspase-3. As shown in Fig. 5B, caspase-3 activity increased in ALA-hx-PDT-treated cells depending on time after irradiation. In addition, apoptosis-related proteins such as cleaved caspase-3/-7 and PARP increased in ALA-hx PDT cells (Fig 5C), whereas the expression level of Bcl-2, an anti-apoptotic protein, decreased. These results suggested that ALA-hx-PDT induced apoptotic cell death. However, a previous study with same photosensitizer demonstrated that ALA-hx-PDT could induce necrosis in

normal and malignant cancer cell lines.[15] In this study, we used 5 μM of ALA-hx, a relatively lower dose, but they used 100 μM of ALA-hx. Even though we did not examine the pattern of cell death dependent of dose, the dose of ALA-hx can be one of reason for the difference of the cell death pattern. Another possibility can be the difference of cell type. To clarify this further study should be designed.

In conclusion, ALA-hx was the more efficacious inducer of PpIX synthesis, compared with same concentration of ALA in oral cancer cell line. Photodynamic efficacy showed a better correlation with the amount of PpIX synthesis as well as CPO mRNA level. PDT with ALA-hx has provided evidence for the strong inhibition of cell viability and induction of apoptosis in oral cancer cells as well as production of ROS, hence demonstrating its potential as a target for oral cancer therapy.

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# 저작물 이용 허락서

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| 논문제목 | 한글: 구강암에서 5-Aminolevulinic Acid-Hexenyl Ester의 광역학치료 효과<br>영문: Photodynamic Efficacy with a Novel Hexenyl Ester of 5-Aminolevulinic Acid in Oral Cancer Cells | | | | |

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

2009년 5 월 29 일

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