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Isolation, identification, and apoptosis activity of the photosensitizer methyl pheophorbide A from *Perilla frutescens* leaves

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Abstract

Photodynamic therapy (PDT) is a promising cancer treatment with fewer side effects, and it eliminates tumors in target tissues with reactive oxygen species produced by photosensitizers (PS) and light. In this study, we isolated methyl pheophorbide A, which induces photodynamic cell death in the U937 and SK-HEP-1 cells, from perilla leaves. Its potential as a material for the development of a new PS was also evaluated. The methyl pheophorbide A is a dark green porphyrin compound isolated from methanol extract of perilla leaves. Apoptosis occurred was methyl pheophorbide A treated and irradiated with light, and there was no significant change where light not treated. In both U937 and SK-HEP-1 cells, apoptotic body, vesicle formation, and DNA ladder were confirmed in the light-irradiated. The caspase-3/7 activity an important factor in apoptosis, was $101.50 \pm 14.24\%$ when treated with $0.25 \mu\text{g/ml}$ methyl pheophorbide A in U937, and $91.32 \pm 16.23\%$ when treated with $1.00 \mu\text{g/ml}$ in SK-HEP-1 cells. The apoptotic phenomenon appeared more strongly when the methyl pheophorbide A concentration was increased with irradiating light. This study demonstrates the isolation, identification, and phototoxic activity of methyl pheophorbide A in perilla leaves. We expect that this study will be useful in the search for PS candidates using natural products.

Keywords: Apoptosis, Cell toxicity, Perilla, Photosensitizer, Pheophorbide

Introduction

Photodynamic therapy (PDT) is being attended as a new cancer treatment strategy, since it has less severe side effects compared to conventional cancer treatments. PDT is a medical treatment that uses reactive oxygen species (ROS) production of a photosensitizer (PS). It is based on the fact that PS reacts with oxygen when it reaches to an excited state by light in a specific wavelength, and produces oxidants in tissues to induce cell death [1]. PDT has advantages compared to traditional treatments, such

as high tumor selectivity, low side effect, non-invasive nature, and high possibility of repeated therapy without cumulative toxic effects [2].

Cell death mechanisms by PDT are as follows: induction of cytotoxicity due to reactive oxygen species, cell death from activation of an immune response, and local depletion of oxygen and nutrients according to vascular shutdown [3]. The PS currently in use are the Photofrin[®] and Photogem[®] based on hematoporphyrin derivatives (HpD). These PS have disadvantages that the available ranges of light wavelength are limited, and the light must be blocked during the PS metabolism except for the light treatment to cancer cells [4]. Therefore, it is necessary to search for various candidate compounds that can solve these problems and to develop a new PS [5].

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Perilla frutescens is an annual herb belonging to the Lamiaceae family, and is originated from East Asia countries. The leaves, stems, and seeds of these family plants are widely used in folk medicine. Various phytochemicals exist in seeds, leaves, and stems of *P. frutescens*, and 271 natural substrates are reported [6]. It is classified into hydrophilic (anthocyanins, phenolic acids, and flavonoids) and hydrophobic (phytosterols, policosanols, and tocopherols) according to its chemical properties. It has been reported various biological activities such as anti-cancer, anti-inflammatory, anti-allergic, antioxidant, and neuroprotective effects [7–10]. Also, there are functional studies with perilla leaves extracts on cell death due to apoptosis in various cancer cells such as hepatoma HepG2 cells, leukemia HL-60 cells, and colon adenocarcinoma DLD-1 cells [10–12].

We, therefore, tested availability of perilla leaves as a source of an improved PS. A solvent extract was obtained from the perilla leaves and isolated a compound which have photodynamic activity. Here we report the identification of methyl pheophorbide A from series of chromatography analyses, which have not been reported previously.

Materials and methods

Solvent extraction

Dried, powdered perilla leaves were extracted with 10 volumes of methanol in a glass container at 150 rpm for 3 h. The extraction was repeated twice and the extract filtered with filter paper (5C. 110 mm, Advantec, Tokyo RoshiKaisha, Ltd., Tokyo, Japan). The filtrate was concentrated with a rotary vacuum evaporator (EYELA N-1000, Japan).

Isolation of photosensitizer

Open column chromatography with normal phase silica gel (Merck, Darmstadt, Germany) and thin layer chromatography (TLC) with TLC Silica gel 60 RP-18 F254s (Merck, Darmstadt, Germany) were used to separate PS. Chromatography separation techniques were applied to separate in PS from methanol extract of perilla leaves. The extract was applied to open column chromatography, and eluted with hexane: ethyl acetate (from 1:0, 5:1, 5:2, 5:3 to 5:4) gradients. The primary 105 fractions were identified by the TLC and the similar R_f (retention factor) values were divided into nine groups (Additional file 1: Fig. S1B). The fractions with similar R_f values were combined and tested PDT-induced apoptosis for U937 and SK-HEP-1 cells. Successive two open column chromatography and a TLC were performed, and a single compound was separated (Additional file 1: Fig. S1C). Liquid chromatography analysis was performed to confirm the purity of the separated compound using

Waters 600 HPLC system and photodiode array detector (Waters, USA). The LC grade methanol and ethyl acetate (Fisher, USA) were used as mobile phase. The column using LiChrospher silica-60 ($5\ \mu\text{m} \times 250\ \text{mm} \times 4.6\ \text{mm}$, Merck, Germany). The gradient elution program of methanol (solvent A) and ethyl acetate (solvent B) was as follows: 0–5 min, 20% B; 5–20 min, 20–80% B; 20–23 min 80–100% B; 23–25 min 100% B. The flow rate was 0.5 ml/min and the detection wavelength was 390 nm. NMR and FAB-Mass analyses were performed to identify the structure of the separated compound.

Structure identification

1D-NMR (^1H -NMR, ^{13}C -NMR) and 2D-NMR (Homo-Cosy, HSQC, HMBC) were conducted using Agilent 600 MHz NMR system (Santa Clara, CA, USA) by dissolving the compound in Deuterated chloroform (CDCl_3 , Aldrich, USA) for NMR to identify the structure of the compound. Chemical shifts are reported in δ (ppm). FAB-mass spectroscopy (JMS 700, Jeol, Japan) was conducted to determine the molecular weight of the compound.

Cell line and cell culture

U937 and SK-HEP-1 cells were received from the Korean Cell Line Bank (KCLB, KOREA). U937 and SK-HEP-1 cells were cultured in RPMI and DMEM medium, respectively. Cell culture was conducted with DMEM medium (Dulbecco's modified eagle) and RPMI 1640 medium (Roswell Park Memorial Institute). FBS (Fetal Bovin Serum) and trypsin–EDTA were purchased from Hyclone (Logan, USA), and penicillin–streptomycin was purchased from Sigma Aldrich (ST. Louis, USA). Medium consisted of 10% (v:v) FBS and 1% (v:v) penicillin–streptomycin. Cells were incubated at 37 °C with 5% CO_2 in a humidified atmosphere. Cells were passaged every 36 h to maintain an appropriate number.

Change in the cell morphology

Changes of cell morphology were observed in the light and dark condition. ADCL was used as a mixture of actinomycin D (Sigma-Aldrich, USA) and colcemid (Sigma-Aldrich, USA). The cells were seeded in 48 well plate at 2×10^5 cells/ml in 0.2 ml and incubated for 12 h. The methyl pheophorbide A dissolved in DMSO (with and without light), 0.1 mM ADCL (the positive control), and DMSO (the negative control) were treated to observe the PDT-induced apoptosis. After 4 h incubation, plate of light condition was irradiated at 3250 Lux for 10 min with daylight fluorescent lamps (wavelengths: 440, 550, and 620 nm). The plate of dark condition was incubated

under light blocked condition at all time. The changes in cell morphology were observed with an IX51 inverted microscope (Olympus, Japan) at 200 × magnification. The pictures were captured by the iSolution Image analysis software.

Cell viability assay

The cell viability was determined by a Cell viability Glo 2.0 assay kit (Promega, USA). Briefly, cells were seeded at a density of 2×10^5 cells/ml in 96 well white plate. After 4 h of incubation, separated compound were treated with different concentrations in each well. After inducing photodynamic activity, cells were mixed with an equal amount of CellTiter-Glo 2.0 reagent for 10 min. Luminescence was measured with a GloMax™ Multi microplate multimode reader.

Assay of caspase-3/7 activity

Caspase activity was measured using a Caspase-Glo 3/7 assay kit (Promega, USA). Cells were seeded at a density of 2×10^5 cells/ml in 96 well white plate and incubated for 4 h. The methyl pheophorbide A was added at different doses to each well. After inducing photodynamic activity, an equal volume of Caspase-Glo solution reagent was added, plates were shaken for 1 h, and luminescence measured with a GloMax™ Multi microplate multimode reader (Promega, USA).

DNA fragmentation assay

To observe DNA fragmentation, which is the biochemical hallmark of apoptosis, cells were seeded at a density of 1×10^6 cells/ml in 6 well plate and incubated for 4 h. The methyl pheophorbide A, ADCL, and DMSO were then added. After inducing photodynamic activity, cells were harvested and washed with ice-cold PBS. Cell pellets were suspended in cell lysis solution (Promega, USA) at 4 °C for 10 min and centrifuged at 4 °C, 14,000 rpm for 20 min. After collection, the supernatant was incubated with 2 µl of RNase A (10 mg/ml, Ambion) and 10 µl of proteinase K (20 mg/ml, Roche) at 37 °C for 1 h, separately. DNA was precipitated with nine volumes of isopropyl alcohol at 4 °C overnight and centrifuged at 14,000 rpm for 20 min at 4 °C. Pellets were dissolved with 0.5% TE buffer. Electrophoresis was performed on 1.6% agarose containing ethidium bromide.

Statistical analysis

All experiments were conducted with three replicates. Statistical analysis was performed using IBM SPSS Statistics 26. The different groups between light and dark conditions were evaluated by two-way ANOVA and Duncan's multiple range test ($p < 0.05$). Data are expressed as mean ± SD (standard deviation).

Results

Separation and identification of methyl pheophorbide

A from *P. frutescens* leaves

We obtained 288.13 g of methanolic extract from 1.2 kg of dried perilla leaves powder. The PS from perilla leaves was successfully separated with series of chromatography techniques (Additional file 1: Fig. S1A). The primary 105 fractions were obtained by the TLC and the similar R_f values were divided into nine groups (Additional file 1: Fig. S1B). Successive two open column chromatography and a TLC were performed to obtain a single compound (Additional file 1: Fig. S1C). As a result, a single peak was confirmed with HPLC at R_t 6.079 min (Additional file 1: Fig. S1D). The separated compound showed a similar UV spectrum with chlorophyll, a representative compound having a phytyl group (Additional file 1: Fig. S2). The single peak compound was further identified with NMR. The $^1\text{H-NMR}$ spectrum is shown in Table 1. The four methine group signals appeared at 8.559 (20-H, s), 9.386 (5-H, s), 9.522 (10-H, s), and 7.98 (3^1-H , dd) ppm, respectively, showing structural similarity with chlorophyll having porphyrin rings [13, 14]. In contrast, the compound did not have a phytyl group compared to chlorophyll which shows multiple signals at around 0.5–1 ppm.

Table 1 $^1\text{H-NMR}$ chemical shift of methyl pheophorbide A (600 MHz, CDCl_3)

Position	Compound (ppm)
2^1-H (CH_3)	3.402 (s)
3^1-H	7.98 (dd, $J = 11.6, 18$)
3^2-H (H trans)	6.29 (dd, $J = 1.2, 18$)
3^2-H (H cis)	6.18 (dd, $J = 1.2, 11.6$)
5-H	9.386 (s)
7^1-H (CH_3)	3.233 (s)
8^1-H (CH_2)	3.659 (q, $J = 7.6$)
8^2-H (CH_3)	1.698 (t, $J = 7.6$)
10-H	9.522 (s)
12^1-H (CH_3)	3.687 (s)
13^2-H	6.251 (s)
13^5-H (CH_3)	3.875 (s)
17-H	4.230 (dd, $J = 2.4, 9.6$)
17^1-H (CHH)	2.614 (m), 2.457 (m)
17^2-H (CHH)	2.412 (m), 2.213 (m)
17^5-H (CH_3)	3.570 (s)
18-H	4.452 (q, $J = 7.2$)
18^1-H (CH_3)	1.809 (d, $J = 6.8$)
20-H	8.559 (s)
21, 23-NH	– 1.65

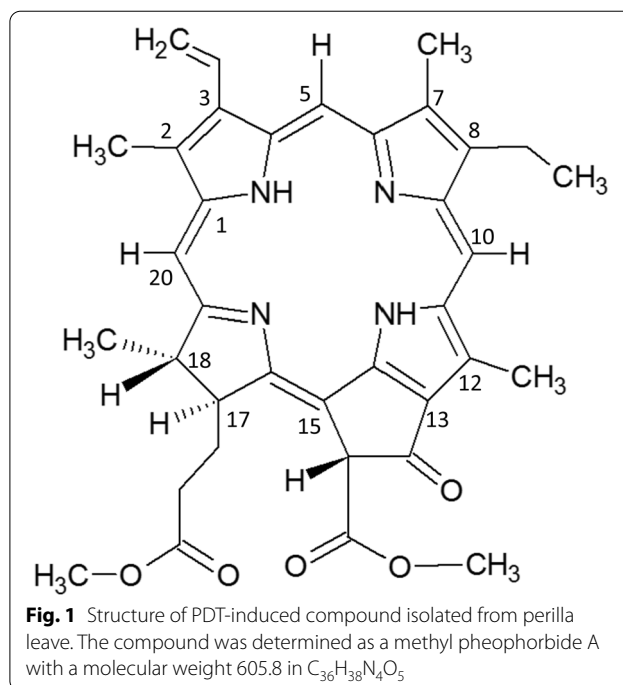
Magnesium ions are present in the center of the porphyrin ring of chlorophyll and since these ions are weak in structural bonding, they are easily eliminated, forming NH bond. In the $^1\text{H-NMR}$ spectrum of the separated compound, NH signal at -1.65 ppm was detected, showing an absence of magnesium ions. The hydrogen bindings to 3^1 and 3^2 -vinyl were detected in 3^1-H (7.98, dd), 3^2-H (H trans, 6.29, d), and 3^2-H (H cis, 6.18, d). The 7C-CH_3 signal appeared as a doublet at 3.22 ppm, indicating that it is a porphyrin compound with the basic structure of chlorophyll a, whereas chlorophyll b has 7C-CHO .

In the $^{13}\text{C-NMR}$ chemical shift (Table 2) of the separated material, the keto carbon [$\delta 189.603(\text{C-}13^1)$], the carbonyl carbon [$\delta 169.56(\text{C-}13^3)$, $\delta 173.324(\text{C-}17^3)$], the methine bridge carbon [$\delta 93.118(\text{C-}20)$, $\delta 97.560(\text{C-}5)$, $\delta 104.454(\text{C-}10)$], the vinyl carbon [$\delta 128.890(\text{C-}3^1)$, $\delta 122.800(\text{C-}3^2)$] signal was similar to chlorophyll a and the previously reported compounds [5]. The carbon signal of the phytol group at 30~40 ppm did not appear in the spectrum of the separated compound, and it was consistent with the $^1\text{H-NMR}$ result.

In addition, FAB-MS analysis was performed to measure the molecular weight, and a fragment signal of $[\text{M-H}]^+$ 605.8 was confirmed (Additional file 1: Fig. S8), resulting in a molecular weight of 605.8. The compound isolated from perilla leaves by combining NMR and MS data was identified as a methyl pheophorbide A, a dark

Table 2 $^{13}\text{C-NMR}$ chemical shift of methyl pheophorbide A (600 MHz, CDCl_3)

Position	Compound (ppm)	Position	Compound (ppm)
1	142.057	12 ¹	12.091
2	131.861	13	128.91
2 ¹	12.091	13 ¹	189.603
3	136.205	13 ²	64.723
3 ¹	128.890	13 ³	169.56
3 ²	122.800	13 ⁵	52.857
4	136.297	14	149.655
5	97.560	15	105.190
6	155.676	16	161.191
7	136.526	17	51.096
7 ¹	11.241	17 ¹	29.357
8	145.243	17 ²	23.053
8 ¹	19.460	17 ³	173.324
8 ²	17.400	17 ⁵	51.670
9	151.004	18	50.092
10	104.454	18 ¹	22.685
11	137.943	19	172.176
12	129.081	20	93.118



green porphyrin compound with a molecular weight of 605.8 (Fig. 1).

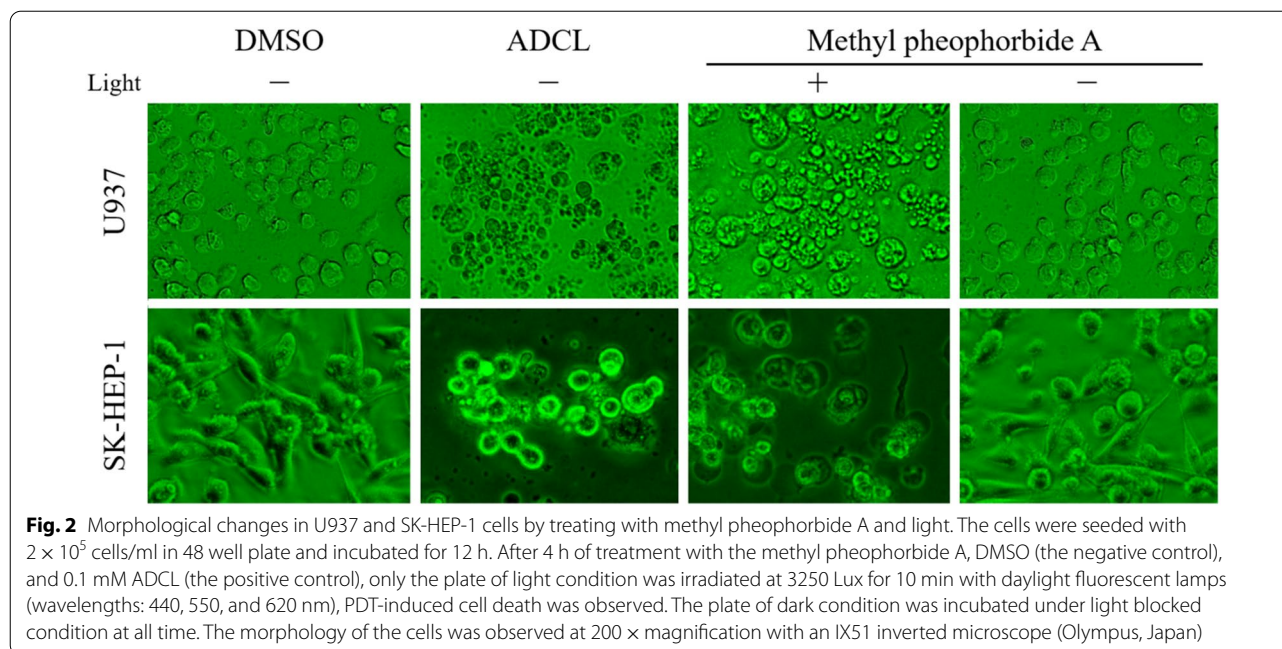
Changes in the cell morphology due to photodynamic activity

To test photodynamic activity of the separated methyl pheophorbide A, apoptosis was observed with two cell lines. The U937 and SK-HEP-1 are originated from histiocytic lymphoma and hepatic adenocarcinoma, respectively and showing different cell growth habitats. U937 cell shows suspension monocyte morphology whereas, SK-HEP-1 cells are epithelial on a culture plate in normal condition.

The methyl pheophorbide A treatment without light did not change cell morphology. However, methyl pheophorbide A with light treatment induced apoptosis with the apoptotic body and vesicle formation in both cell lines similar to 0.1 mM ADCL treatment, the positive control (Fig. 2).

Cell viability by methyl pheophorbide A with light irradiation

The degree of apoptosis was quantified with luminescent cell viability assay for methyl pheophorbide A and light treatment. In U937 cells, ADCL, which induces apoptosis, showed cell viability of $9.79 \pm 0.09\%$, $10.80 \pm 0.16\%$ in light and dark conditions, respectively (Fig. 3A). When methyl pheophorbide A was treated at various concentrations and irradiated with light, cell viability rates of



10.34 ± 0.79% (0.25 µg/ml), 14.28 ± 1.73% (0.50 µg/ml), 0.35 ± 0.03% (1.00 µg/ml), and 0.26 ± 0.05% (2.00 µg/ml) were shown, and most of the cells were observed to die. The cell viability under dark condition was 74.36 ± 1.90% (0.25 µg/ml), 92.54 ± 1.24% (0.50 µg/ml), 82.01 ± 2.66% (1.00 µg/ml), 72.11 ± 4.27% (2.00 µg/ml) similar to the cell viability 84.47 ± 8.70% (the light condition), 92.13 ± 3.57% (the dark condition) of DMSO (the negative control).

The cell viability concentration-dependently decreased by methyl pheophorbide A and light treatment of SK-HEP-1 cells 85.68 ± 1.02% (0.25 µg/ml), 93.16 ± 9.07% (0.50 µg/ml), 16.24 ± 5.16% (1.00 µg/ml), 1.37 ± 0.43% (2.00 µg/ml) (Fig. 3B). The cell viability in dark conditions with methyl pheophorbide A was 78.54 ± 1.89% ~ 89.41 ± 1.39%, and there was no significance with DMSO.

Caspase-3/7 activity in PDT-induced apoptosis

The activity of caspase-3/7, effector caspases, was measured which induces apoptosis by activating nuclease. Caspase is classified into an initiator caspase (caspase-2, -8, -9, and -10) and effector caspase (caspase-3, -6, -7). Effector caspases are known to be the end causatives of apoptosis. The RLU value was calculated as the relative ratio (%) of ADCL (the positive control). When U937 cells were treated with methyl pheophorbide A and irradiated with light, caspase activity was measured as 101.50 ± 14.24% (0.25 µg/ml), 71.31 ± 6.41% (0.50 µg/ml), 22.28 ± 4.29% (1.00 µg/ml), and 4.27 ± 0.26% (2.00 µg/ml). In dark condition, caspase activity was measured

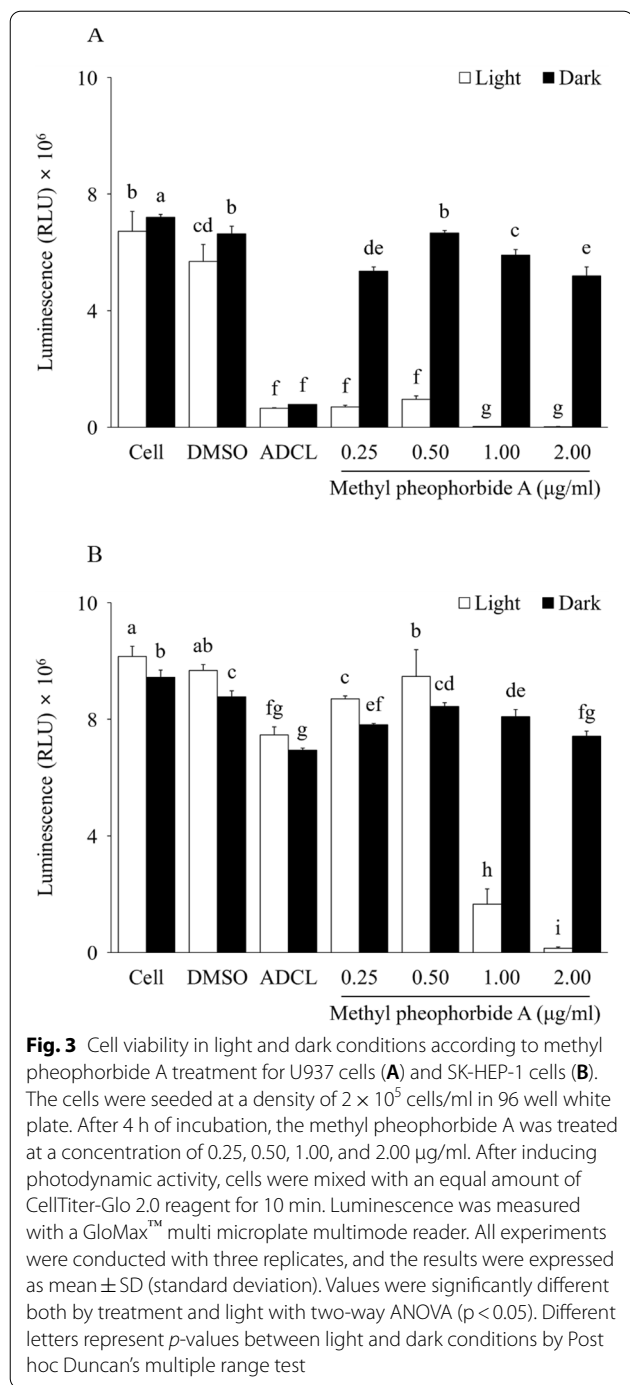
by 18.89 ± 0.65% (0.25 µg/ml), 6.48 ± 0.43% (0.50 µg/ml), 10.19 ± 2.81% (1.00 µg/ml), and 9.61 ± 2.77% (2.00 µg/ml) (Fig. 4A). In dark conditions, there was no significant difference according to the concentration of methyl pheophorbide A. However, the light condition was concentration-dependently decreased caspase activity. The decrease in concentration-dependent activity is presumed to be due to the rapid inactivation of enzymes due to rapid cell death. Cell viability after treatment with 1.00 µg/ml, 2.00 µg/ml of methyl pheophorbide A was 0.35 ± 0.03%, 0.26 ± 0.05%, and most of the cells died.

In SK-HEP-1 cells light condition, caspase activity was 29.22 ± 1.03% (0.25 µg/ml), 30.95 ± 2.79% (0.50 µg/ml), 91.32 ± 16.23% (1.00 µg/ml), and 12.41 ± 1.12% (2.00 µg/ml). In dark condition, caspase activity was measured by 18.48 ± 0.61% (0.25 µg/ml), 19.21 ± 3.46% (0.50 µg/ml), 20.39 ± 4.23% (1.00 µg/ml), and 24.73 ± 1.73% (2.00 µg/ml) (Fig. 4B). The highest caspase activity was shown at 91.32 ± 16.23% in methyl pheophorbide A 1.00 µg/ml treated light irradiated group, but no significant difference was found in other concentration treatments.

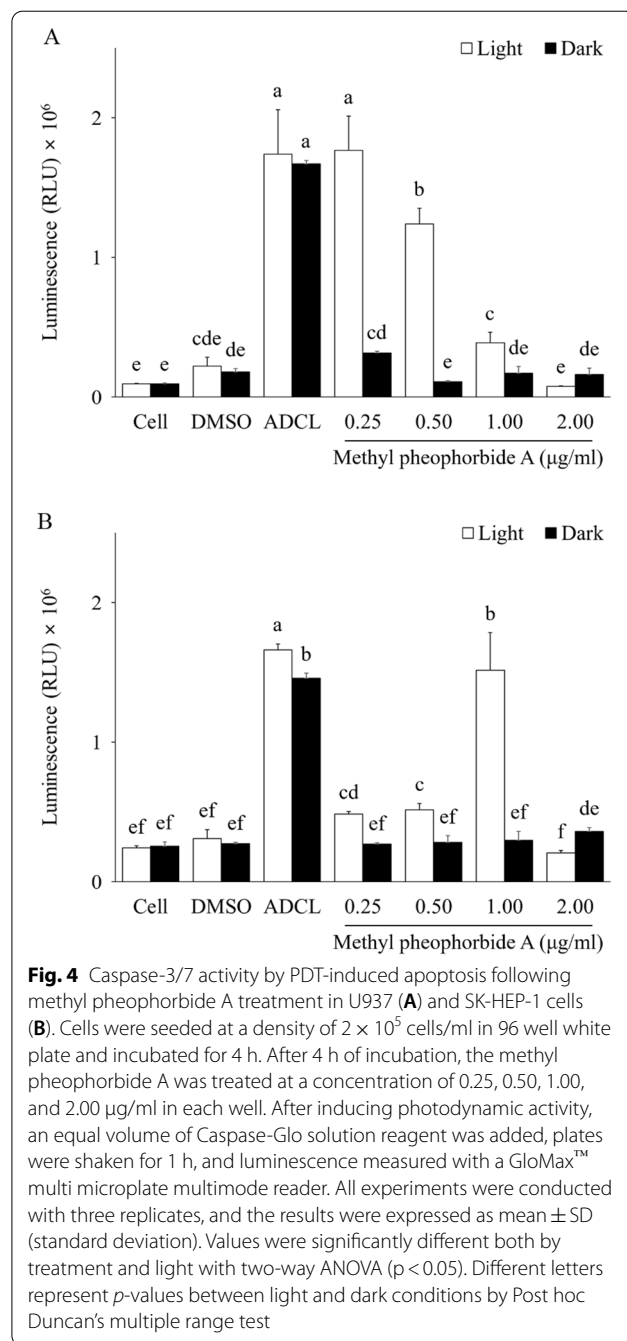
DNA fragmentation by PDT-induced apoptosis

DNA fragmentation is one of the major features of cell death caused by apoptosis. When apoptosis is induced, DNA fragmentation occurs and DNA ladder can be observed. The ADCL (the positive control) treatment lead DNA fragmentation independent to irradiation, whereas DMSO did not.

In the methyl pheophorbide A and light irradiation, the DNA ladder was observed in both cell lines. In dark

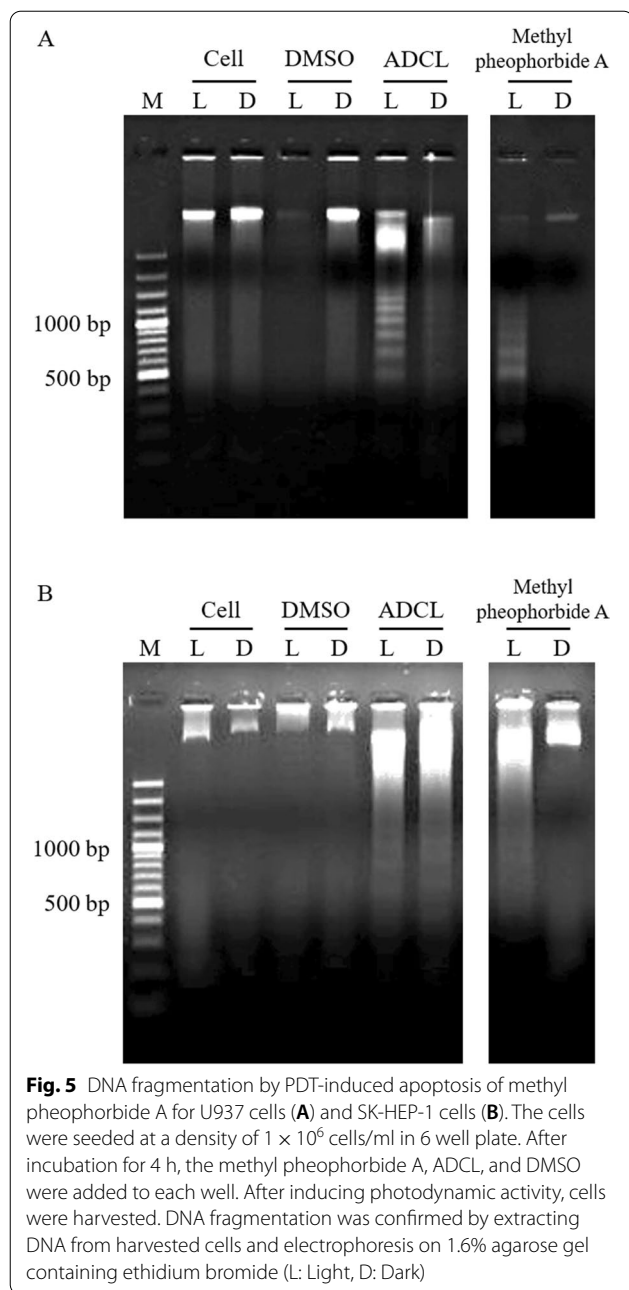


conditions, the DNA ladder was not observed like DMSO (the negative control) treatment (Fig. 5). Therefore, cell death induced by methyl pheophorbide A shows characteristics such as DNA ladders, apoptotic bodies, and vesicles, suggesting that cell death is results of apoptosis.



Discussion

The PDT research aims to find a PS with minimal cytotoxicity in the dark conditions of tissues, a higher concentration in diseased tissues than in healthy tissues, and a higher efficiency generating singlet oxygen and ROS [15]. Photofrin®, a PS widely used in clinics, is effective in various types of cancer, but induces a long-standing cutaneous photosensitivity, and has the longest absorption wavelength of 630 nm, which is limited



in treating deep or larger tumors. Longer wavelengths (>650 nm) of light are more transmissive and can be used to treat deep or larger tumors. Therefore, the new PS development is focused on finding materials with a long wavelength ranges between 650 and 850 nm [16].

The separated methyl pheophorbide A from perilla leaves in this study have a typical porphyrin structure similar to chlorophyll a. The previously reported porphyrin compounds with photocytotoxicity have a lactone

structure between ^{13}C and ^{13}C , with or without OH group on ^{13}C [5]. The spectrums of the lactone containing porphyrin have ^{13}C signal at 189.603 ppm and ^{13}C signal at 64.723 ppm, however, the methyl pheophorbide A in this study did not show such signals indicating that it has not a lactone structure.

Pheophorbides are plant metabolites related to chlorophyll and are used as major photoactive compounds in PDT [17]. Anti-cancer activities and photocytotoxicities of pheophorbide a, pyropheophorbide a, and pheophytin-a have been isolated from various plant species including *Piper penangense*, *Phaeanthus ophthalmicus*, and *Zanthoxylum ailanthoides* Sieb. & Zucc has been reported [18–20]. This the first study on the photocytotoxicity of methyl pheophorbide A isolated from *P. frutescens* leaves to our best knowledge. Cytotoxic activity, anticancer activity, antimetastasis effects, and antioxidant response element dependent transcriptional regulation of methyl pheophorbide A isolated from various natural products have been reported [21–23].

PDT-induced cell death is mainly resulted by apoptosis among the three major reasons of cell death; apoptosis, autophagy, and necrosis [3]. Two major pathways of PDT-induced apoptosis are the death receptor-mediated extrinsic pathway and the mitochondria-mediated or intrinsic pathway. Cell surface receptors of tumor necrosis factor genes are activated by initiator caspase-8 via adaptor and scaffolding protein in death receptor-mediated extrinsic pathway. Mitochondria-mediated or intrinsic pathway started with a mitochondrial functional disturbance which induces a release of cytochrome c. Cytosolic cytochrome c binds to Apaf-1, which induces Apaf-1 oligomerization in the presence of dATP. This complex, called an apoptosome, activates the initiator caspase-9. In both pathways, activation of the initiator caspase (caspase-8 or caspase-9) leads to the activation of the effector caspase (caspase-3, -6, -7) showing caspase-3/7 is a good indicator for observing apoptosis [24].

In the methyl pheophorbide A treated with the light condition, cell death was induced in U937 and SK-HEP-1 cells with apoptotic body and vesicle formation, which are characteristics of apoptosis. According to light irradiation, caspase-3/7 activity was increased to a similar level as the positive control ADCL. The DNA fragmentation, one of the characteristics of apoptosis, was observed in methyl pheophorbide A treatment with light. In U937 and SK-HEP-1 cells, cell viability and caspase-3/7 activity were found to be dependent on the methyl pheophorbide A concentration. The cell death pathway is due to various mechanisms [25, 26]. The difference in amount and efficiency of PS localization in diseased tissues with types of PS compound and cells should be further investigated.

In this study, methyl pheophorbide A has been isolated from perilla leaves with series of chromatography. This compound is a dark green porphyrin compound, $C_{36}H_{38}N_4O_5$, and a molecular weight of 605.8 $[M-H]^+$. The methyl pheophorbide A induces cell death depending on light, and exhibits a characteristic of apoptosis. This compound has the potential to be utilized as a PS which would have apoptosis activity and availability of broad light wavelength. It is important to discover various candidate compounds with physiological activity from natural products. We expect that this study will be useful in the search for PS candidates using natural products.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-022-00719-5>.

Additional file 1: Fig. S1. Procedure of the methyl pheophorbide A isolation from *Perilla frutescens* leaves. (A) Strategy of chromatographies for the methyl pheophorbide A isolation. (B) Rf patterns of the fractions from primary column chromatography with thin layer chromatography. TLC was visualized with visible light (top), UV short wave (middle), UV long wave (bottom). (C) Final purification of methyl pheophorbide A with TLC which was visualized with visible light (left), UV short wave (middle), UV long wave (right). (D) HPLC analysis for the purified methyl pheophorbide A. **Fig. S2.** Scanning of absorbance (y-axis) of continuous light. **Fig. S3.** 1H NMR spectrum ($CDCl_3$, 600 MHz) of methyl pheophorbide A. **Fig. S4.** ^{13}C NMR spectrum ($CDCl_3$, 600 MHz) of methyl pheophorbide A. **Fig. S5.** COSY NMR spectrum ($CDCl_3$, 600 MHz) of methyl pheophorbide A. **Fig. S6.** HMBC NMR spectrum ($CDCl_3$, 600 MHz) of methyl pheophorbide A. **Fig. S7.** HSQC NMR spectrum ($CDCl_3$, 600 MHz) of methyl pheophorbide A. **Fig. S8.** FAB-MS spectrum of methyl pheophorbide A.

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Author contributions

KKK: Experimental guidance and overall management. 1st author JYH: Separation of photosensitizer. 2nd author GY: Pella cultivation. 3rd author HHB: Pella cultivation and statistical processing. 4th author YSG: Pella cultivation and statistical processing. 5th author YJK: Apoptosis assay. 6th author KML: DNA ladder. 7th author COH: Structure identification. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Competing interests

Not applicable.

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