Photodiagnosis and Photodynamic Therapy 21 (2018) 121–127

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Novel potential photodynamic therapy strategy using 5-Aminolevulinic acid for ovarian clear-cell carcinoma

Toshiya Teshigawara[a], Mika Mizuno[b,c,*], Takuya Ishii[d], Yuya Kitajima[d], Fumi Utsumi[a], Jun Sakata[a], Hiroaki Kajiyama[a], Kiyosumi Shibata[a], Masahiro Ishizuka[d], Fumitaka Kikkawa[a]

[a] Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Tsuruma-cho 65, Showa-ku, Nagoya, 466-8550, Japan
[b] Department of Gynecology, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya, 464-8681, Japan
[c] Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya, 464-8681, Japan
[d] SBI Pharmaceuticals Co., Ltd., Izumi Garden Tower 20F, Roppongi 1-6-1, Minato-ku, Tokyo, 106-6020, Japan

ARTICLE INFO

Keywords:
5-aminolevulinic acid
Protoporphyrin IX
Photodynamic therapy
Ovarian cancer
Clear-cell carcinoma

ABSTRACT

Background: Photodynamic therapy (PDT) is known as a minimally invasive treatment for cancer. 5-Aminolevulinic acid (ALA) is a precursor of the photosensitizing agent protoporphyrin IX (PpIX). Patients with ovarian clear-cell carcinoma (CCC) have poorer prognoses than those of patients with other histological CCC types. We evaluated the efficacy of ALA-PDT on CCC cells in vitro.

Methods: We used seven human CCC cell lines to measure the cytotoxicity of ALA-PDT. PpIX production in cancer cells was measured using a micro-plate reader. Quantitative real-time PCR was performed to assess the mRNA levels of genes involved in the accumulation of PpIX in cancer cells. Additionally, we measured the enhancement in cytotoxicity with the use of an ABCG2 inhibitor.

Results: We found that three cell lines were highly sensitive to ALA-PDT. In contrast, one cell line was resistant to ALA-PDT. The cytotoxicity of ALA-PDT varied among CCC cell lines. The IC50 values of ALA-PDT for the CCC cell lines had a wide range (30–882 μM). The cytotoxicity of ALA-PDT was correlated with the intracellular PpIX accumulation. The cell lines sensitive to ALA-PDT expressed PEPT1 (an ALA uptake transporter). The cell line resistant to ALA-PDT expressed ABCG2 (a PpIX export transporter). In the resistant cell line, a combination treatment with both ALA and an ABCG2 inhibitor resulted in the promotion of cytotoxic sensitivity.

Conclusion: The present study revealed the efficacy of ALA-PDT against CCC with chemoresistance in vitro.

1. Introduction

It is well known that ovarian cancer is the leading cause of tumor-related death in gynecological cancer [1]. Early-stage ovarian cancer has few subjective symptoms because the ovaries are in the pelvis. Thus, approximately 50% of women with the disease are in an advanced stage when diagnosed. Nearly all patients with advanced disease undergo cytoreductive surgical resection followed by chemotherapy [2]. Clear-cell carcinoma (CCC) is a histological subtype that accounts for 23.4% of all ovarian cancer in Japan [3]. Ovarian CCC has a low response rate to platinum-based chemotherapy; thus, it is associated with a poorer prognosis than that of serous [4,5] and endometrioid adenocarcinoma [6].

Photodynamic therapy (PDT) is an effective and minimally invasive treatment for cancer. It has been reported that some factors are involved in the efficacy of PDT. PDT is generally based on the photochemical interaction of a photosensitizer that can accumulate specifically in cancer tissue with light and oxygen. The type of photosensitizer, light dose, and photosensitizer concentration are also factors related to the effect. Several authors have described that PDT has two types of reactions. The type I reaction results in hydrogen or electron transfer between the photosensitizer and substrate to produce free radicals. These radicals react with oxygen to yield superoxide radical anions. In the type II reaction, singlet oxygen is produced through an energy transfer process with ground state oxygen and the return of the photosensitizer to the ground state [7]. As a representative mechanism, PDT results in the generation of reactive oxygen species (ROS) containing singlet oxygen, which induces apoptosis [8]. There are also reports indicating that an immune response is involved in the effect of PDT. PDT can induce tumor death and microangiopathy, which lead to the increased expression of pro-inflammatory cytokines and the activation of immune cells [9].

In Japan, Photofrin® and Laserphyrin® have been used as photosensitizers in PDT covered by insurance for lung and esophageal cancer.

* Corresponding author at: Department of Gynecology, Aichi Cancer Center Hospital 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan.
E-mail address: mmizuno@aichi-cc.jp (M. Mizuno).

https://doi.org/10.1016/j.pdpdt.2017.11.013
Received 24 July 2017; Received in revised form 20 October 2017; Accepted 27 November 2017
Available online 28 November 2017
1572-1000/

The clinical application of PDT is expected to expand to include other cancer types. In terms of PDT, only Photofrin® with an excimer-dye laser was approved for use in treating cervical intraepithelial neoplasia (carcinoma in situ); however, this type of laser is no longer produced, and the maintenance support period ended in March 2017. These photosensitizers have a side effect, i.e., phototoxic skin reactions [10].

In this study, we used 5-aminolevulic acid (ALA), which is water soluble and administered orally [11]. The substance is an endogenous amino acid, and it is usually synthesized from glycine and succinyl CoA in mitochondria. In addition, ALA is not a photosensitizer, it is the precursor of protoporphyrin IX (PpIX) in the heme metabolic pathway. ALA is converted to the active photosensitizer PpIX within cells [12]. PpIX is excreted from the body after 24–48 h via the heme metabolic pathway; therefore, patients do not need to avoid light for a long time [13]. In cancer cells, the irradiation of PpIX with red light at a wavelength of 635 nm induces the generation of ROS, such as singlet oxygen and free radicals, which subsequently cause apoptosis and necrosis [14,15]. Although ingested exogeneous ALA is converted to heme in normal cells, cancer cells specifically accumulate PpIX without metabolizing it to produce heme. In addition, the intracellular accumulation of PpIX was found to involve the solute carrier (SLC) transporter and ATP-binding cassette (ABC) transporter [16–18].

In the present study, we examined the cytotoxicity of ALA-PDT in CCC cells. Furthermore, we explored the expression levels of transporters involved with the cytotoxicity of ALA-PDT and the accumulation of PpIX. The possible application of this modality as a therapy for CCC is proposed.

2. Materials and methods

2.1. Biochemicals and cell culture

ALA hydrochloride was obtained from SBI Pharmaceuticals Co., Ltd. (Tokyo, Japan). We used seven human ovarian CCC cell lines in this study: ES2, TOV21G, KOC7C, OVTOKO, RMG1, RMG2 and OVMANA. ES2 and TOV21G cells were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). KOC7C cells were a generous gift from Dr. Junzo Kigawa (Tottori University, Tottori, Japan). OVTOKO, RMG1, RMG2, and OVMANA cells were a generous gift from Dr. Kiyoshi Hasegawa (Fujita Health University, Aichi, Japan). The cell lines were maintained in RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum and penicillin-streptomycin in a humidified atmosphere of 5% CO_2 at 37 ° C.

2.2. Light sources for PDT

A light-emitting diode (LED) was used as a source of light at a wavelength of 631 nm. A power density of 17.4 mW/cm^2 was used in this study.

2.3. Cytotoxicity of ALA-PDT

We evaluated the cytotoxicity of ALA-PDT on CCC cells. Cell viability was measured by Cell Counting Kit 8 assay (CCK-8; Dojindo Laboratories, Kumamoto, Japan). ES2, TOV21G, KOC7C and OVTOKO cells were seeded at a density of 1×10^4 cells in 100 µl of media in 96-well plates, and RMG1, RMG2 and OVMANA cells were seeded at a density of 2×10^4 cells in 100 µl of media in 96-well plates. The cells were cultured in an atmosphere with 5% CO_2 at 37 ° C for 24 h. The culture media was replaced with fresh media containing ALA at 0–1000 µM. Cells were incubated with ALA in the dark for 4 h. Then, the cells were washed in phosphate-buffered saline (PBS), and the PBS was replaced with fresh media prior to light irradiation. Irradiation with 631-nm light was performed for 600 s. After irradiation, the cells were incubated at 37 ° C with 5% CO_2 for 24 h. The cells were washed in PBS before the culture media was replaced with 100 µl of 10% CCK-8 reagent. After the cells were incubated at 37 ° C with 5% CO_2 for 30 min, the absorbance at 450 nm was measured using a micro-plate reader (Tecan infinite M200PRO, Zurich, Switzerland). We also prepared a control group without irradiation to evaluate the cytotoxicity of ALA.

2.4. PpIX accumulation in cancer cells incubated with ALA

We evaluated the intracellular and extracellular accumulation of PpIX in CCC cells incubated with ALA. ES2, TOV21G, KOC7C and OVTOKO cells were seeded at a density of 1×10^4 cells in a 100-µl volume in 96-well black wall plates, and RMG1, RMG2 and OVMANA cells were seeded at a density of 2×10^4 cells in a 100-µl volume in 96-well black wall plates. These cells were cultured at 37 ° C with 5% CO_2 for 24 h. The culture media was replaced with fresh media containing ALA at various concentrations. The final concentration of ALA ranged from 0 to 1000 µM. The cells were incubated with ALA in the dark for 4 h. Extracellular PpIX was measured in the supernatant. Intracellular PpIX was measured in cells processed with 100 µl of 1% sodium dodecyl sulfate after being washed twice with PBS. The resulting fluorescence was measured using a micro-plate reader (Tecan Infinite M200 PRO). We used an excitation wavelength of 405 nm and emission wavelengths of 632 nm and 635 nm for measuring intracellular and extracellular PpIX accumulation, respectively. The measured values of PpIX accumulation were corrected using protein measurements. The protein concentration was measured using a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions.

2.5. Detection of mRNA levels by quantitative real-time PCR (qRT-PCR)

Total RNA extraction was performed with an RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Extracted RNA was immediately stored at −80 ° C. The RNA concentration was determined using a spectrophotometer (Beckman Coulter, Pasadena, CA, USA). Transcription was performed using a High-capacity RNA-to-cDNA kit (Applied Biosystems, Waltham, MA, USA) according to the manufacturer's instructions. To obtain complementary DNA (cDNA), 2 µg of RNA was used, and the resulting cDNA was stored at −20 ° C. The RNA levels of PEPT1 (SLC15A1), PEPT2 (SLC15A2), TAUT (SLC6A6), GAT2 (SLC6A13), PAT1 (SLC36A1), ABCG2, ferrochelatase (FECH) and β-actin were determined by qRT-PCR (Applied Biosystems® Step One Plus™) with a SYBR Select Master Mix (Applied Biosystems®). β-Actin was used as the internal control, and 20 ng of cDNA was used for qRT-PCR. The cycling conditions consisted of a 2-min hot start at 95 ° C, followed by 40 cycles of denaturation at 95 ° C for 15 s, annealing at 60 ° C for 60 s, extension at 95 ° C for 15 s, and then a final inactivation at 95 ° C for 15 s. Relative quantification was performed using the ΔΔCT method. The specific primers for each gene are shown in Table 1. All experiments were performed in triplicate.

2.6. The enhancing effect of ALA-PDT and the accumulation of intracellular PpIX via an ABCG2 inhibitor

We examined the role of ABCG2 in the cytotoxicity induced by ALA-PDT and the accumulation of PpIX. We investigated the inhibitory effect of ABCG2 with an ABCG2-specific inhibitor, fumitremorgin C (FTC). ES2 cells were incubated with ALA (0–1000 µM) for 4 h in the absence or presence of 10 µM FTC according to previously reported methods with some modifications [19,20]. The PDT treatment was followed by cell viability measurements. For the PpIX measurements, ES2 cells were incubated with ALA (0–1000 µM) and FTC (10 µM) in the dark for 4 h. We then measured the intracellular and extracellular PpIX levels according to the abovementioned procedure.

Table 1
The specific primers for each gene.

Primer	Sequence	Primer	Sequence
PEPT1	F:GGCAGGCCAGTTCAGCAA R:AGCAACGCGGCAAATAGAA	hPAT1	F:TCTGCCGCAGGCTGAATAAA R:AGTCCACAACACGTCTTCCC
PEPT2	F:CAGCAGGGTTCACGATGGA R:GGTCCGGCTGAAGCACAA	FECH	F:AGTCTAACATCAGGAAGCCGA R:AGTGTCATGAGGTCTCGGTC
TAUT	F:AGGAGCTCCCAAACAAAGCA R:GGAGGTTTTCCCTCAGCCTC	ABCG2	F:TTTACGGCTTTGCAGCATAATG R:TCTTCGCCAGTACATGTTGCAT
GAT2	F:GGCAGCAGCTTCACTAAGGT R:GGTGCCATCTTCCTCCTTCT	β-actin	F:ACAGAGCCTCGCCTTTGC R:CGCGGCGATATCATCATCCA

F, forward R, reverse

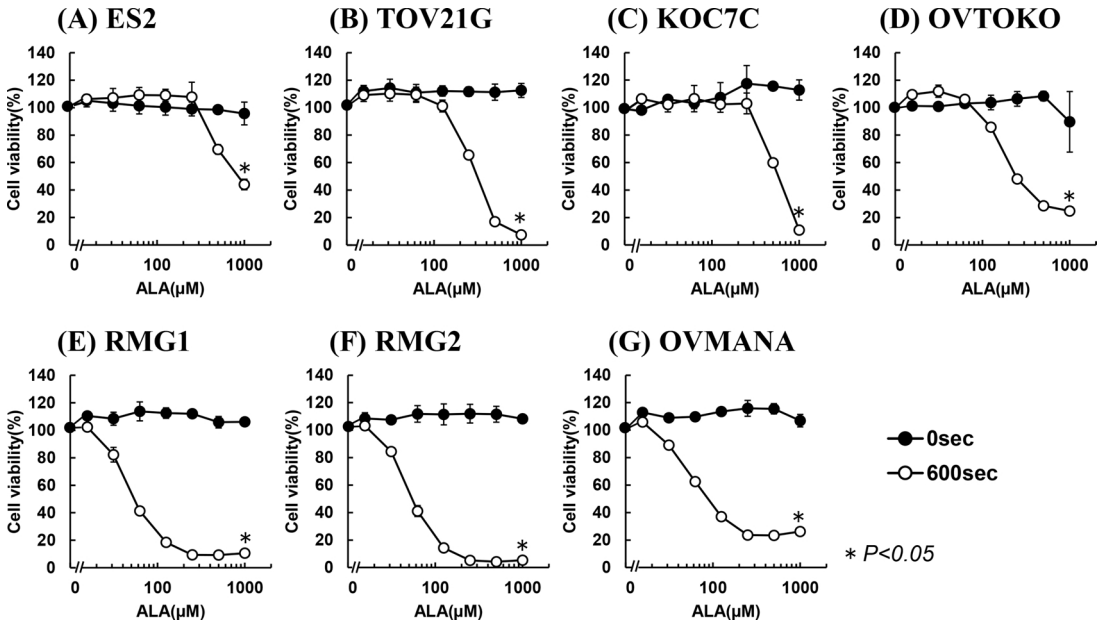

Fig. 1. Cell growth inhibition effect of ALA-PDT. CCC cell lines (ES2, TOV21G, KOC7C, OVTOKO, RMG1, RMG2 and OVMANA) were incubated with different concentrations of ALA (0–1000 μM) and irradiated for 0 or 600 s.WST8 assay was used to detect the cytotoxicity of ALA-PDT. Data are expressed as the mean ± S.D. in multi-replicated experiments ($n = 3$). The statistical significance of differences between paired groups is indicated by $^*P < 0.05$. (A): ES2, (B): TOV21G, (C): KOC7C, (D): OVTOKO, (E): RMG1, (F): RMG2, (G): OVMANA.

3. Results

3.1. Cytotoxicity of ALA-PDT on CCC cells

This study demonstrates the different cytotoxic effects of ALA-PDT among CCC cell lines (Fig. 1). The results indicated that ALA-PDT was effective against CCC cells in a dose-dependent manner. RMG1, RMG2 and OVMANA cells were more sensitive to ALA-PDT than the other cell lines. The IC50 values for RMG1, RMG2 and OVMANA cells were calculated to be 57, 30, 54 μM ALA, indicating that these three cell lines were sensitive to ALA-PDT. In contrast, ES2 cells were resistance to ALA-PDT. The IC50 value for ES2 was calculated to be 882 μM ALA. Table 2 shows the IC50 values for the CCC cell lines in ALA-PDT. The treatments without subsequent light exposure exerted less cytotoxicity in the CCC cells. The CCC cell lines could be classified into the three subgroups with respect to ALA-PDT: the sensitive group (RMG1, RMG2 and OVMANA cells), the resistant group (ES2 cells) and the intermediate group (TOV21G, KOC7C and OVTOKO cells).

3.2. Intracellular and extracellular PpIX accumulation after incubation with ALA

Based on the idea that the cytotoxicity of ALA-PDT depends on the intracellular accumulation of PpIX, we next examined the accumulation of PpIX synthesized from ALA. The intracellular accumulation of PpIX in the sensitive group (RMG1, RMG2 and OVMANA cells) was detected at approximately 60 μM ALA (Fig. 2). For the other cell lines, intracellular PpIX was detected at approximately 250 μM ALA (Fig. 2). Among all CCC cell lines, the intracellular accumulation of PpIX was elevated in an ALA dose-dependent manner. Intracellular PpIX accu-

Table 2
The IC50 values of ALA-PDT for each CCC cell line.

Cell line	ES2	TOV21G	KOC7C	OVTOKO	RMG1	RMG2	OVMANA
IC50 value (μM)	882	330	857	244	56	56	97
PpIX (nmol/mg protein) *	0.41	0.91	0.98	0.42	0.6	0.83	0.43

*ALA, 1 mM

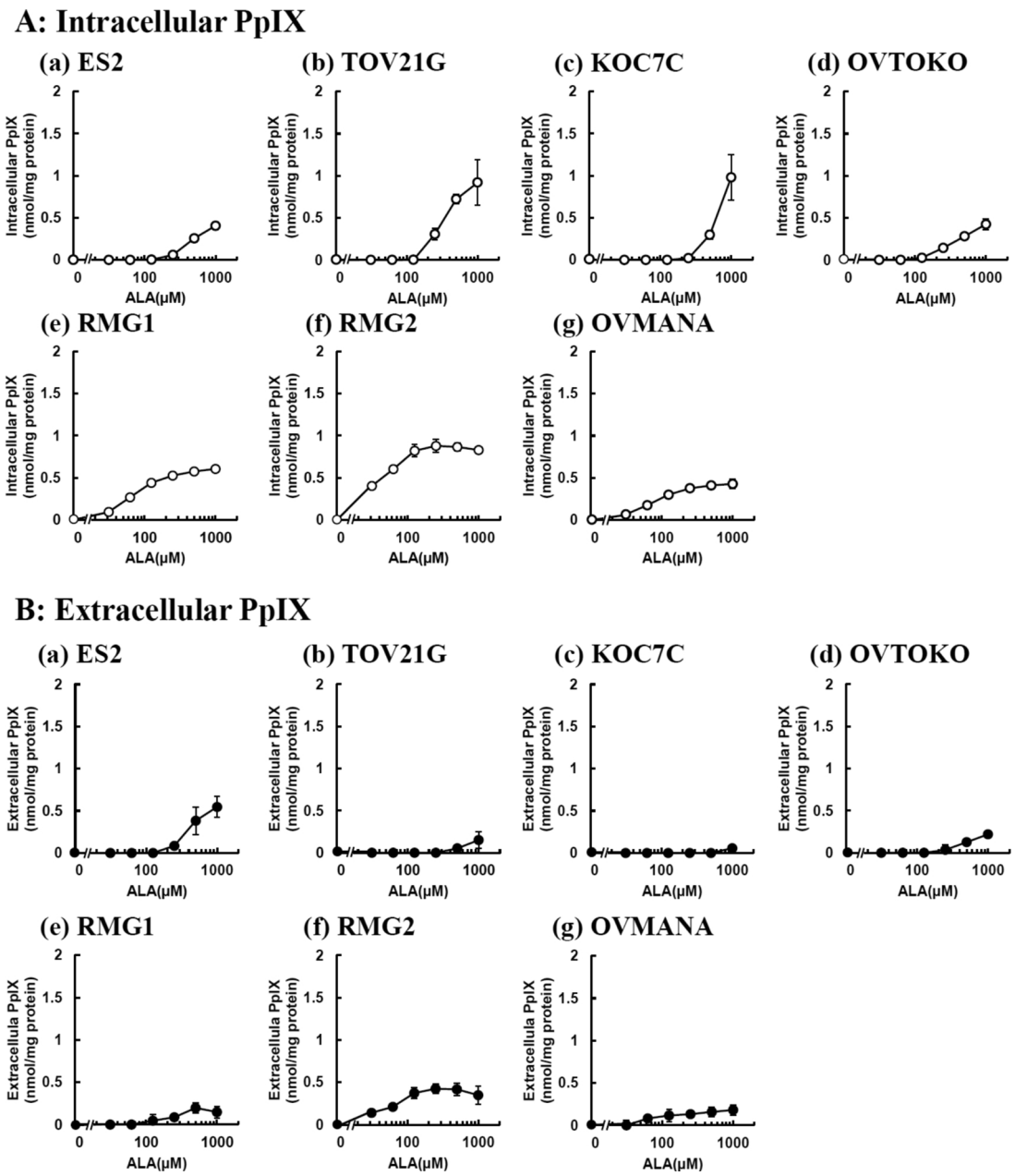

Fig. 2. Extracellular and intracellular PpIX accumulation after incubation with ALA (0–1000 μM) for 4 h. Data are expressed as the mean ± S.D. of multi-replicated experiments (n = 3). A: intracellular PpIX, B: extracellular PpIX, (a): ES2, (b): TOV21G, (c): KOC7C, (d): OVTOKO, (e): RMG1, (f): RMG2, (g): OVMANA.

mulation was correlated with the cytotoxicity of ALA-PDT in each cell line. ES2 cells stimulated by 1000 μM ALA produced the highest level of extracellular PpIX among all CCC cell lines.

3.3. Expression of transporters involved in PpIX accumulation

We subsequently examined the expression of genes involved in PpIX accumulation in CCC cells. The relative mRNA levels of PEPT1, PEPT2, TAUT, GAT2, PAT1, FECH and ABCG2 in CCC cell line were calculated, as shown in Fig. 3. Reportedly, ALA is transported into cells via PEPT1, PEPT2, TAUT, GAT2 and PAT1 [16,20,21]. The expression levels of PEPT1, PEPT2 and GAT2 were greater in RMG1, RMG2 and OVMANA cells than in the other cell lines. These three cell lines were sensitive to ALA-PDT (Fig. 1). The expression of PEPT1 was not detected in the other cell lines, indicating that PEPT1 expression might contribute to the cytotoxicity of ALA-PDT in RMG1, RMG2 and OVMANA cells. TAUT and PAT1 expression was detected in all CCC cell lines. These two transporters might be involved in the cytotoxicity of ALA-PDT in CCC cells. PpIX was transported extracellularly via ABCG2. The highest mRNA level of ABCG2 was detected in ES2 cells, which were resistant to ALA-PDT. Although a previous study has shown that FECH is a key enzyme for the intracellular accumulation of PpIX [22], we found no differences in the expression of FECH among the cell lines observed. PEPT1, TAUT, PAT1 and ABCG2 might play important roles in CCC cell subjected to ALA-PDT.

3.4. Enhanced cytotoxicity of ALA-PDT using an ABCG2 inhibitor

As previously mentioned, ES2 exhibited the highest expression of ABCG2. We investigated whether an ABCG2-specific inhibitor, fumitremorgin C (FTC), could improve the efficacy of ALA-PDT and the ALA-induced PpIX accumulation in ES2 cells (Fig. 4). Concurrent incubation with ALA and FTC resulted in increased sensitivity to ALA-PDT. In ES2 cells incubated with 1000 μM and 10 μM FTC, the

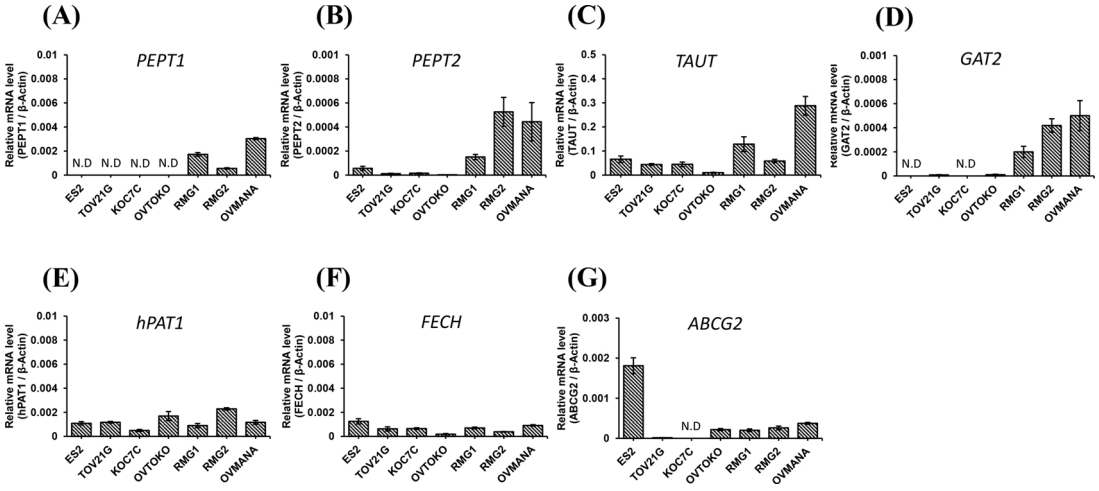

Fig. 3. mRNA expression levels of porphyrin biosynthesis-related transporters and porphyrin biosynthesis enzymes. (A)-(G) mRNA levels of PEPT1, PEPT2, TAUT, GAT2, PAT1, FECH, ABCG2 and β-actin as determined by real-time PCR with specific primers. β-Actin was used as the internal control. Data are expressed as the mean ± S.D. of multi-replicated experiments (n = 3). N.D., not detected, indicating a threshold cycle (CT) cut-off > 35.

intracellular accumulation of PpIX increased by two-fold. Thus, the ABCG2 inhibitor contributed to the increased intracellular accumulation of PpIX in ES2 cells expressing ABCG2. In other CCC cell lines, which expressed low levels of ABCG2, we confirmed that the combination treatment with ALA and FTC did not enhance the cytotoxicity of ALA-PDT or the intracellular accumulation of PpIX (Suppl. Figs. 1, 2). These results suggested that ABCG2 was an important factor of the cytotoxicity of ALA-PDT.

4. Discussion

Many reports comparing CCC to other histological types of epithelial ovarian cancer show that in advanced stages, CCC has poorer chemosensitivity than other types [4–6]. Recent reports have indicated the beneficial effects of molecular targeted therapies, such as PIK3CA-targeting drugs [23] and immune checkpoint inhibitors [24], for CCC; however, the results of the relevant clinical trials are not yet available. Thus, new treatment strategies for these CCC patients are needed. This is the first study to demonstrate the efficacy of ALA-PDT against CCC using various cell lines.

In this study, we showed that while ALA-PDT exerted the highest efficacy against RMG1, RMG2, and OVMANA CCC cells, some cell lines were resistant, e.g., ES2 cells. The IC50 value of ALA-PDT in ES2 cells was approximately 30-times higher than that in RMG2 cells. The intracellular accumulation of PpIX was correlated with the effects of ALA-PDT in these CCC cell lines. Thus, we speculated that the efficacy of ALA-PDT was reflected by the intracellular PpIX concentration. We next investigated the association between the efficacy of ALA-PDT and the expression of CCC cell transporters involved in the accumulation of PpIX. PEPT1 (an ALA uptake transporter) has a wide substrate

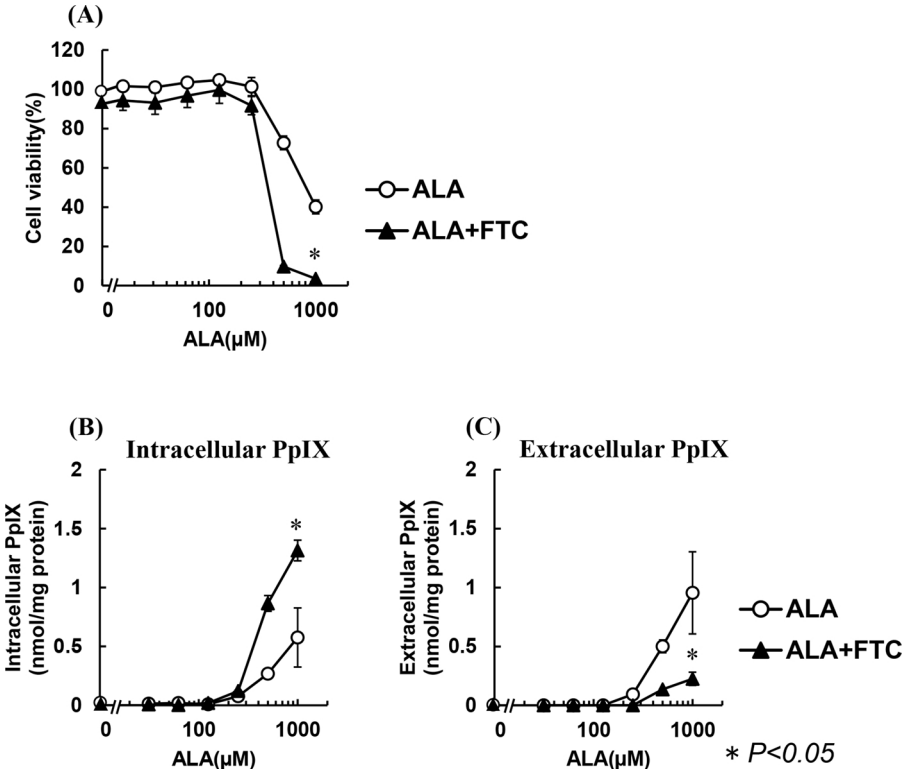

Fig. 4. Enhancing effect of an ABCG2-specific inhibitor (FTC) on ALA-based phototoxicity and PpIX accumulation. (A): Cytotoxic effect of ALA-PDT with an ABCG2-specific inhibitor (FTC) in ES2 cells. ES2 cells were incubated ALA (0–1000 μM) with 10 μM FTC for 4 h. ES2 cells were irradiated for 600 s. (B), (C): ES2 cells were incubated with ALA (0–1000 μM) for 4 h in the absence or presence of 10 μM FTC. Intracellular and extracellular PpIX accumulation were determined. Data are expressed as the mean ± S.D. of multi-replicated experiments (n = 3). The statistical significance of differences between paired groups is indicated by *P < 0.05.

specificity for numerous compounds in normal tissues, and the expression of this transporter is found in epithelial cells of the small intestine, kidneys, and bile duct. Regarding the expression of transporters in CCC cells, the gene expression of PEPT1 was observed to be higher only in RMG1, RMG2 and OVMANA cells, which were more sensitive to ALA-PDT than ES2 cells. Therefore, it was suggested that the expression of PEPT1 might contribute to the cytotoxicity of ALA-PDT in CCC cells. In contrast, the highest level of ABCG2 (a PpIX export transporter) expression was found in ES2 cells, which were more resistant to ALA-PDT than were RMG1, RMG2 and OVMANA cells. These results suggest that high PEPT1 (ALA uptake transporter) expression and low ABCG2 (porphyrin export transporter) expression are related to the efficacy of ALA-PDT. Hagiya's study showed similar results in gastric cells in vitro [20]. Furthermore, we demonstrated that the ABCG2 inhibitor FTC improved the cytotoxicity of ALA-PDT and the intracellular PpIX accumulation in ES2 cells. FTC cannot be used in an actual clinical situation because of its neurotoxicity [25]. Nevertheless, these results indicate that increasing PpIX accumulation in cancer cells using ABCG2 inhibitors is very important for clinical applications. ABCG2 has been indicated to be expressed in cancerous cells and to contribute to multidrug resistance by transporting anti-tumor drugs out of cells [26,27]. ABCG2 has a wide substrate specificity for structurally diverse compounds. ABCG2 substrates include anticancer drugs, such as doxorubicin, topotecan, irinotecan and SN-38 [28]. Recently, it has been reported that anticancer drugs, such as gefitinib and liposomal doxorubicin (Doxil®), exert a synergistic effect in combination with ALA-PDT [29,30]. These drugs may have the potential to enhance the cytotoxicity of ALA-PDT.

According to some reports, other treatments combined with ALA increased the cytotoxic effects of ALA-PDT and the accumulation of PpIX. For example, in cervical cancer, gold nanoparticles used as a drug delivery system increased PpIX production [31]. Another study showed that other LED wavelengths, such as green and violet, exerted greater anti-tumor effects than did red [32]. For the clinical application of ALA-PDT, it is necessary to improve its anti-tumor efficacy. The depth of tissue invasion by PDT is superficial, as it is less than 5 mm [33]. As PDT treatment cannot exert a sufficient effect, it is difficult to treat advanced peritoneal dissemination with PDT alone.

Photodynamic diagnosis (PDD) using ALA has already been applied for diagnosing brain and bladder tumors in general practice. Some clinical trials have reported using ALA-PDD for the peritoneal dissemination of gastric, colorectal and ovarian cancer [34–36]. Accurate staging via ALA-PDD may contribute to improving treatment outcomes. Peritoneal dissemination is also a characteristic of metastasis in ovarian cancer [37]. We frequently find adhesions between tumors and neighboring organs or small but macroscopic peritoneal nodules during ovarian cancer surgery. In these cases, it is difficult to determine whether the cancer is an inflammatory change. In CCC with chemoresistance, it is required to remove tumors thoroughly. It is expected that ALA-PDT in combination with ALA-PDD will become a useful therapeutic approach for chemoresistant ovarian cancer.

The most popular precursor photosensitizers for PDT are ALA and methyl ALA. Yokoyama et al. have reported the good results of PDT with methyl ALA in vivo using 5 ovarian cancer cell lines (3 serous tumor, 1 clear-cell, and 1 mucinous tumor) [38]. The results of PDT in the serous tumor cells showed significant tumor shrinkage compared with the controls. The study also indicated that high expression levels of the glutathione transferase Omega-1 gene, which is involved in the conversion of PpIX into heme in cells, are associated with increased sensitivity to PDT. As we mentioned above, in general, serous adenocarcinoma is chemosensitive, while CCC is chemoresistant. Our study shows the efficacy of PDT against some CCC cells and an association between the effect of ALA-PDT and the gene expression of transporters in seven CCC cell types. Of the two precursors, ALA is hydrophilic and can be administered orally, whereas methyl ALA is lipophilic and cannot be administered orally. As orally administered ALA is an approved optical imaging agent used to enhance the intraoperative detection of brain and bladder tumors, ALA may be useful more methyl ALA; however, methyl ALA may penetrate tissues more deeply with a higher specificity [39]. In addition, it has been reported that compared with methyl ALA, ALA leads to three-fold greater PpIX accumulation [40].

In summary, we have shown that ALA-PDT has potential as an effective method for treating ovarian cancer. In cancer cells resistant to ALA-PDT, an ABCG2 inhibitor improved the cytotoxicity of ALA-PDT. The present study suggests that PEPT1 and ABCG2 might be useful indicators for predicting the efficacy of ALA-PDT. Further investigation is needed to examine the anti-tumor efficacy of ALA-PDT in vivo using experimental xenograft models and potentially clinical trials. In the clinical application of ALA-PDT for ovarian cancer with peritoneal dissemination and unresectable tumors, we hope that the combination of surgery and ALA-PDT may be a treatment strategy. We consider that irradiation of a pelvis with micro peritoneal dissemination following tumor removal achieve the desired therapeutic effect and prevent recurrence. Furthermore, it might be possible to predict the efficacy of ALA-PDT via the personal gene profiling of surgical samples.

Conflicts of interest

The authors have no conflicts of interest to declare.

Funding

Grants-in-Aid for Scientific Research; some funds of the Grants-in-Aid program were transferred to the Japan Society for the Promotion of Science from Monbusho (now Monbu Kagakusho [Ministry of Education, Culture, Sports, Science and Technology, MEXT]) from 1 FY1999.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.pdpdt.2017.11.013.

References

[1] Foundation for Promotion of Cancer Research, Cancer Statics in Japan, (2015) (accessed 12 Nov 2016). http://ganjoho.jp/data/reg_stat/statistics/brochure/2015/cancer_statistics_2015_fig_E.pdf.
[2] S.A. Cannistra, Cancer of the ovary, N. Engl. J. Med. 351 (2004) 2519–2529.
[3] T. Saito, H. Katabuchi, Annual report of the Committee on Gynecologic Oncology, Japan Society of Obstetrics and Gynecology: patient annual report for 2013 and treatment annual report for 2008, J. Obstet. Gynaecol. Res. 42 (2016) 1069–1079.
[4] M. Mizuno, F. Kikkawa, K. Shibata, H. Kajiyama, K. Ino, M. Kawai, T. Nagasaka, S. Nomura, Long-term follow-up and prognostic factor analysis in clear cell adenocarcinoma of the ovary, J. Surg. Oncol. 94 (2006) 138–143.
[5] T. Sugiyama, T. Kamura, J. Kigawa, N. Terakawa, Y. Kikuchi, T. Kita, M. Suzuki, I. Sato, K. Taguchi, Clinical characteristics of clear cell carcinoma of the ovary: a distinct histologic type with poor prognosis and resistance to platinum-based chemotherapy, Cancer 88 (2000) 2584–2589.
[6] M. Takano, Y. Kikuchi, N. Yaegashi, K. Kuzuya, M. Ueki, H. Tsuda, M. Suzuki, J. Kigawa, S. Takeuchi, H. Tsuda, T. Moriya, T. Sugiyama, Clear cell carcinoma of the ovary: a retrospective multicentre experience of 254 patients with complete surgical staging, Br. J. Cancer 94 (2006) 1369–1374.
[7] D. Phillips, Photodynamic therapy, Sci. Prog. 77 (Pt 3–4) (1993) 295–316.
[8] S.B. Brown, E.A. Brown, I. Walker, The present and future role of photodynamic therapy in cancer treatment, Lancet Oncol. 5 (2004) 497–508.
[9] P.C. Kousis, B.W. Henderson, P.G. Maier, S.O. Gollnick, Photodynamic therapy enhancement of antitumor immunity is regulated by neutrophils, Cancer Res. 67 (2007) 10501–10510.
[10] S. Mimura, Y. Ito, T. Nagayo, M. Ichii, H. Kato, H. Sakai, K. Goto, Y. Noguchi, H. Tanimura, Y. Nagai, S. Suzuki, Y. Hiki, Y. Hayata, Cooperative clinical trial of photodynamic therapy with photofrin II and excimer dye laser for early gastric cancer, Lasers Surg. Med. 19 (1996) 168–172.
[11] M. Ishizuka, F. Abe, Y. Sano, K. Takahashi, K. Inoue, M. Nakajima, T. Kohda, N. Komatsu, S. Ogura, T. Tanaka, Novel development of 5-aminolevurinic acid (ALA) in cancer diagnoses and therapy, Int. Immunopharmacol. 11 (2011) 358–365.
[12] J.C. Kennedy, R.H. Pottier, Endogenous protoporphyrin IX, a clinically useful

photosensitizer for photodynamic therapy, J. Photochem. Photobiol. B 14 (1992) 275–292.
[13] Q. Peng, T. Warloe, K. Berg, J. Moan, M. Kongshaug, K.E. Giercksky, J.M. Nesland, 5-Aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges, Cancer 79 (1997) 2282–2308.
[14] B.B. Noodt, K. Berg, T. Stokke, Q. Peng, J.M. Nesland, Apoptosis and necrosis induced with light and 5-aminolaevulinic acid-derived protoporphyrin IX, Br. J. Cancer 74 (1996) 22–29.
[15] N.L. Oleinick, R.L. Morris, I. Belichenko, The role of apoptosis in response to photodynamic therapy: what, where, why, and how, Photochem. Photobiol. Sci. 1 (2002) 1–21.
[16] T.T. Tran, A. Mu, Y. Adachi, Y. Adachi, S. Taketani, Neurotransmitter transporter family including SLC6A6 and SLC6A13 contributes to the 5-aminolevulinic acid (ALA)-induced accumulation of protoporphyrin IX and photodamage, through uptake of ALA by cancerous cells, Photochem. Photobiol. 90 (2014) 1136–1143.
[17] B. Mackenzie, D.D. Loo, Y. Fei, W.J. Liu, V. Ganapathy, F.H. Leibach, E.M. Wright, Mechanisms of the human intestinal H+-coupled oligopeptide transporter hPEPT1, J. Biol. Chem. 271 (1996) 5430–5437.
[18] F. Doring, J. Walter, J. Will, M. Focking, M. Boll, S. Amasheh, W. Clauss, H. Daniel, Delta-aminolevulinic acid transport by intestinal and renal peptide transporters and its physiological and clinical implications, J. Clin. Invest. 101 (1998) 2761–2767.
[19] T. Ogino, H. Kobuchi, K. Munetomo, H. Fujita, M. Yamamoto, T. Utsumi, K. Inoue, T. Shuin, J. Sasaki, M. Inoue, K. Utsumi, Serum-dependent export of protoporphyrin IX by ATP-binding cassette transporter G2 in T24 cells, Mol. Cell. Biochem. 358 (2011) 297–307.
[20] Y. Hagiya, Y. Endo, Y. Yonemura, K. Takahashi, M. Ishizuka, F. Abe, T. Tanaka, I. Okura, M. Nakajima, T. Ishikawa, S. Ogura, Pivotal roles of peptide transporter PEPT1 and ATP-binding cassette (ABC) transporter ABCG2 in 5-aminolevulinic acid (ALA)-based photocytotoxicity of gastric cancer cells in vitro, Photodiagnosis Photodyn. Ther. 9 (2012) 204–214.
[21] S. Frolund, O.C. Marquez, M. Larsen, B. Brodin, C.U. Nielsen, Delta-aminolevulinic acid is a substrate for the amino acid transporter SLC36A1 (hPAT1), Br. J. Pharmacol. 159 (2010) 1339–1353.
[22] Y. Ohgari, Y. Nakayasu, S. Kitajima, M. Sawamoto, H. Mori, O. Shimokawa, H. Matsui, S. Taketani, Mechanisms involved in delta-aminolevulinic acid (ALA)-induced photosensitivity of tumor cells: relation of ferrochelatase and uptake of ALA to the accumulation of protoporphyrin, Biochem. Pharmacol. 71 (2005) 42–49.
[23] M.L. Friedlander, K. Russell, S. Millis, Z. Gatalica, R. Bender, A. Voss, Molecular profiling of clear cell ovarian cancers: identifying potential treatment targets for clinical trials, Int. J. Gynecol. Cancer 26 (2016) 648–654.
[24] J. Hamanishi, M. Mandai, T. Ikeda, M. Minami, A. Kawaguchi, T. Murayama, M. Kanai, Y. Mori, S. Matsumoto, S. Chikuma, N. Matsumura, K. Abiko, T. Baba, K. Yamaguchi, A. Ueda, Y. Hosoe, S. Morita, M. Yokode, A. Shimizu, T. Honjo, I. Konishi, Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer, J. Clin. Oncol. 33 (2015) 4015–4022.
[25] T.S. Garimella, D.D. Ross, J.L. Eiseman, J.T. Mondick, E. Joseph, T. Nakanishi, S.E. Bates, K.S. Bauer, Plasma pharmacokinetics and tissue distribution of the breast cancer resistance protein (BCRP/ABCG2) inhibitor fumitremorgin C in SCID mice bearing T8 tumors, Cancer Chemother. Pharmacol. 55 (2005) 101–109.
[26] R. Januchowski, K. Wojtowicz, P. Sujka-Kordowska, M. Andrzejewska, M. Zabel, MDR gene expression analysis of six drug-resistant ovarian cancer cell lines, Biomed. Res. Int. 2013 (2013) 241763.
[27] H.G. Liu, Y.F. Pan, J. You, O.C. Wang, K.T. Huang, X.H. Zhang, Expression of ABCG2 and its significance in colorectal cancer, Asian Pac. J. Cancer Prev. 11 (2010) 845–848.
[28] Y. Liang, S. Li, L. Chen, The physiological role of drug transporters, Protein Cell 6 (2015) 334–350.
[29] S. Zakaria, A.M. Gamal-Eldeen, S.M. El-Daly, S. Saleh, Synergistic apoptotic effect of Doxil (R) and aminolevulinic acid-based photodynamic therapy on human breast adenocarcinoma cells, Photodiagnosis Photodyn. Ther. 11 (2014) 227–238.
[30] W. Sun, Y. Kajimoto, H. Inoue, S. Miyatake, T. Ishikawa, T. Kuroiwa, Gefitinib enhances the efficacy of photodynamic therapy using 5-aminolevulinic acid in malignant brain tumor cells, Photodiagnosis Photodyn. Ther. 10 (2013) 42–50.
[31] H. Eshghi, A. Sazgarnia, M. Rahimizadeh, N. Attaran, M. Bakavoli, S. Soudmand, Protoporphyrin IX-gold nanoparticle conjugates as an efficient photosensitizer in cervical cancer therapy, Photodiagnosis Photodyn. Ther. 10 (2013) 304–312.
[32] H. Hino, Y. Murayama, M. Nakanishi, K. Inoue, M. Nakajima, E. Otsuji, 5-Aminolevulinic acid-mediated photodynamic therapy using light-emitting diodes of different wavelengths in a mouse model of peritoneally disseminated gastric cancer, J. Surg. Res. 185 (2013) 119–126.
[33] National Cancer Institute, Photodynamic Therapy for Cancer, (2016) (accessed 12 Nov 2016), http://www.cancer.gov/cancertopics/factsheet/Therapy/photodynamic.
[34] N. Koizumi, Y. Harada, Y. Murayama, K. Harada, M. Beika, Y. Yamaoka, P. Dai, S. Komatsu, T. Kubota, D. Ichikawa, K. Okamoto, A. Yanagisawa, E. Otsuji, T. Takamatsu, Detection of metastatic lymph nodes using 5-aminolevulinic acid in patients with gastric cancer, Ann. Surg. Oncol. 20 (2013) 3541–3548.
[35] Y. Kondo, Y. Murayama, H. Konishi, R. Morimura, S. Komatsu, A. Shiozaki, Y. Kuriu, H. Ikoma, T. Kubota, M. Nakanishi, D. Ichikawa, H. Fujiwara, K. Okamoto, C. Sakakura, K. Takahashi, K. Inoue, M. Nakajima, E. Otsuji, Fluorescent detection of peritoneal metastasis in human colorectal cancer using 5-aminolevulinic acid, Int. J. Oncol. 45 (2014) 41–46.
[36] Y. Liu, Y. Endo, T. Fujita, H. Ishibashi, T. Nishioka, E. Canbay, Y. Li, S. Ogura, Y. Yonemura, Cytoreductive surgery under aminolevulinic acid-mediated photodynamic diagnosis plus hyperthermic intraperitoneal chemotherapy in patients with peritoneal carcinomatosis from ovarian cancer and primary peritoneal carcinoma: results of a phase I trial, Ann. Surg. Oncol. 21 (2014) 4256–4262.
[37] F. Utsumi, H. Kajiyama, K. Nakamura, H. Tanaka, M. Mizuno, K. Ishikawa, H. Kondo, H. Kano, M. Hori, F. Kikkawa, Effect of indirect nonequilibrium atmospheric pressure plasma on anti-proliferative activity against chronic chemo-resistant ovarian cancer cells in vitro and in vivo, PLoS One 8 (2013) e81576.
[38] Y. Yokoyama, T. Shigeto, R. Miura, A. Kobayashi, M. Mizunuma, A. Yamauchi, M. Futagami, H. Mizunuma, Differences in the sensitivity of ovarian cancer to photodynamic therapy and the mechanisms for those differences, Oncol. Lett. 13 (2017) 4933–4938.
[39] M.H. Gold, Therapeutic and aesthetic uses of photodynamic therapy part five of a five-part series: ALA-PDT and MAL-PDT what makes them different, J. Clin. Aesthet. Dermatol. 2 (2009) 44–47.
[40] P. Mikolajewska, V. Iani, A. Juzeniene, J. Moan, Topical aminolaevulinic acid- and aminolaevulinic acid methyl ester-based photodynamic therapy with red and violet light: influence of wavelength on pain and erythema, Br. J. Dermatol. 161 (2009) 1173–1179.