Basic Report

Apoptosis-inducing effect of 5-aminolevulinic acid-mediated photodynamic therapy (5-ALA-PDT) on cervical cancer cell lines

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Key words: cervical neoplasm, 5-aminolevulinic acid, photodynamic therapy, apoptosis, cell line

Background and Objective: 5-Aminolevulinic acid-mediated photodynamic therapy (ALA-PDT) is a new ablation treatment for tumors, while its function mechanism in cervical cancer is unclear. This study was to investigate the effects of ALA-PDT on cervical cancer cell lines. Methods: The effects of ALA-PDT on proliferation of eight human cervical cancer cell lines were examined by MTT assay to find out the optimal function parameters of ALA–PDT and the most sensitive cell line. The effect of ALA-PDT on apoptosis of cervical cancer cell line Me180 was investigated by using Annexin V-FITC/PI double staining, Hoechst 33342 staining and May-Grünwald-Giemsa staining. Cell cycle of Me180 cells was observed by flow cytometry with PI staining. The effect of ALA-PDT on the expression of survivin in Me180 cells was detected by Western blot and real-time fluorescence reverse transcription-polymerase chain reaction (RT-PCR). Results: Among the eight cell lines, Me180 cell line was most sensitive to ALA-PDT; 2 mmol/L ALA, 10 J/cm² light dose and 3-hour treatment were the optimal function parameters of ALA–PDT, and the 50% inhibition concentration (IC50) of ALA-PDT under this situation for Me180 cells was 7.28 × 10⁻⁴ mmol/L. ALA-PDT induced apoptosis and G0/G1 phase arrest of Me180 cells, and suppressed the mRNA and protein expression of survivin in Me180 cells. Conclusion: ALA-PDT can inhibit the proliferation and induce apoptosis of cervical cancer cell line Me180 in vitro, which may relate with the suppression of survivin expression.

Currently, cervical cancer looms large increasingly among young people, among whom most are single or not pregnant and thus require the maintenance of fertility. In the meantime, some patients, who are old, cannot tolerate the surgery and chemotherapy, or refuse the surgery, urgently need an effective palliative treatment.

The photodynamic therapy (PDT) with visible light radiation combined with local administration of 5-aminolevulinic acid (5-ALA) recently emerges as a tumor ablation technique. As a new treatment with high selectivity, it has become the fourth anti-tumor therapy, following surgery, chemotherapy and radiotherapy.1 ALA is an endogenous simple five-carbon compound which is synthesized with glycin, succinic acid and coenzyme A by ALA synthase and is insensitive to light radiation. ALA participates in the biological synthesis of hemachrome in vivo. As a precursor of hemachrome, ALA can produce the protoporphyrin IX (PpIX) with high sensitivity to light radiation under the activities of ALA dehydratase and other enzymes. PpIX is a precursor in the last step during the synthesis of hemachrome. In normal circumstances, the biological synthesis of hemachrome is adjusted by the negative feedback mechanism. In other words, the synthesis of ALA is mediated by the quantity of hemachrome in cells, therefore, ALA will not being accumulated excessively in the body. When overdose of exogenous ALA is administered, the modulation mechanism is disrupted and some rapidly proliferated body cells or tumor cells produce a large quantity of PpIX. In such a condition, laser radiation will result in photodynamic reaction which produces cytotoxic substances of 1O2 and other free radicals to kill tumor cells.

The mechanism of ALA-PDT acting on cervical cancer cell lines has not been reported. The present study was to investigate the efficacy of ALA-PDT on cervical cancer in vitro and to clarify the mechanism of ALA-PDT inducing apoptosis of cervical cancer cells, so as to seek a new approach for the treatment of early stage cervical cancer and cervical precancerous lesion and the adjuvant treatment of advanced cervical cancer, and to provide theoretical reference for clinical application.

Materials and Methods

Materials. Main reagents. DMEM, fetal bovine serum and trypsin were all purchased from Gibco Company. ALA, MTT and PI were purchased from Sigma Company. The RNA extraction kit was purchased from QIAGEN Company. Annexin V-FITC/PI was purchased from Beijing Biosea Biotechnology Co., LTD. Survivin rabbit polyclonal antibody and Western blot Luminol Reagent (sc-2048) were purchased from Santa Cruz Company. β-actin
mouse monoclonal antibody, HRP-marked sheep anti-rabbit IgG antibody and sheep anti-mouse IgG antibody were all purchased from Zhongshan Golden Bridge Biotechnology Co., LTD. The PRO-PREPTM protein extracts were from Sai Bosheng Company. The protein quantitative test kit was purchased from Applygen Company. The protein pre-staining marker and the reverse transcription kit were purchased from MBI Company. The primers were synthesized by Invitrogen Company. The SYBR® Premix Ex Taq™kit was purchased from TaKaRa Company.

Main instruments. The Model 6300 CO2 incubator was purchased from NAPCO Company. The DU800 spectrophotometer was bought from Beckman Company. The BHS-313 microscope and IX-70 inverted fluorescence microscope were purchased from Olympus Company. The refrigerated centrifuge was bought from HITACHI Company. The Cytospin 3 cell-centrifugation-smear machine was purchased from Shandon Company. The He-Ne laser was bought from Tsinghua University Laser Technology Company. The fluorescent scanning analyzer was bought from Vistra Fluorescence Company. The flow cytometry was bought from BD Company. The enzyme mark instrument was purchased from Thermo Company. The 7300 Real-Time PCR System and fluorescence PCR System were purchased from ABI Company. The electroforesis system was purchased from Bio-Rad Company. The electroforesis system was purchased from Alpha Innotech Company.

Methods. Cell lines and cell culture. Cervical cancer cell lines Me80 (HTB-33), C-33A (HTB-31), HeLa (CCL-13), Caski (CRL-1550), C4I (CRL-1594), HT3 (HTB-32) and SiHa (HTB-35) were presented by Dr. Takeshi Yamashita from Asahikawa Medical Institute of Basic Medical Sciences, Japanese Academy of Medical Sciences. The cells were cultured in DMEM containing 10% fetal bovine serum and stored in the incubator at 37°C with 5% CO2.

Main instruments. The Model 6300 CO2 incubator was purchased from NAPCO Company. The DU800 spectrophotometer was bought from Beckman Company. The BHS-313 microscope and IX-70 inverted fluorescence microscope were purchased from Olympus Company. The refrigerated centrifuge was bought from HITACHI Company. The Cytospin 3 cell-centrifugation-smear machine was purchased from Shandon Company. The He-Ne laser was bought from Tsinghua University Laser Technology Company. The fluorescent scanning analyzer was bought from Vistra Fluorescence Company. The flow cytometry was bought from BD Company. The enzyme mark instrument was purchased from Thermo Company. The 7300 Real-Time PCR System and fluorescence PCR System were purchased from ABI Company. The electroforesis system was purchased from Bio-Rad Company. The electroforesis system was purchased from Alpha Innotech Company.

Optimal function parameters of ALA-PDT concentration and laser energy and most sensitive cell lines screened by MTT assay. Cervical cancer cells were inoculated in 96-well plates with a concentration of 5 x 10^4/well. At 24 h after the cells were cultured, ALA at final concentrations of was 10, 1, 0.1, 0.01, 0.001, 0.000 1 and 0.000 01 mmol/L was added. Blank control group, ALA alone and 0.000 01 mmol/L was added. Blank control group, ALA alone and radiation alone group were set with 3 wells for each. After the cells were cultured in dark with ALA in the incubator for 6 h, the medium containing ALA was removed and 200 µL medium containing 10% DMEM was added in each well. Single layers of cells were radiated by laser (630 nm) from up to down. The spot diameter was 1 cm and the doses of laser were 10, 20, and 30 J/cm², respectively. After radiation, the medium was changed and the cells were cultured in fresh DMEM in dark for 3 h. Each plate was radiated by laser at a single dose with a spot diameter of 3 cm. The medium was removed, 180 µL fresh drug-free medium and 20 µL MTT solution (5 mg/mL) were added in each well, and cells were cultured at 37 in dark for 4 h. The supernatant was carefully removed, and 150 µL DMSO was added to each well. Then the plates were shaken for 10 min. The absorbance at 570 nm (A570) for each well was measured by a microplate reader. The A570 of each well was subtracted by the background A570 (for medium with MTT but no cells). The A570 of parallel wells were recorded as means. The proliferation inhibition rate = (A570 of control cells - A570 of ALA-treated cells) / A570 of control cells x 100%. The 50% inhibition concentration (IC50) was calculated according to Karbers Method. The calculation was repeated for three times and mean values were recorded.

Optimal function time of ALA-PDT screened by MTT assay. Me180 cells were chosen and radiated by laser (630 nm) at a dose of 10 J/cm². After radiation, the medium was replaced with fresh DMEM medium and the cells were cultured in dark for 1, 2, 3, 4, 6, 12 and 24 h. MTT assay was performed at each time point to screen the optimal function time of ALA-PDT.

Effect of ALA-PDT on apoptosis of Me180 cells detected by flow cytometry with Annexin V-FITC/PI double staining. Me180 cells were inoculated in 6-well plates and cultured for 24 h. After medium replacement, Me180 cells were added with ALA at final concentrations of 0.1, 1 and 2 mmol/L. Blank control group, ALA alone and 0.1, 1 and 2 mmol/L. Blank control group, ALA alone and radiation alone group were set with 3 wells for each. After the cells were cultured in dark for 3 h and 24 h, gathered and cleansed by PBS twice, then suspended in 150 µL Binding buffer, added with 10 µL Annexin V-FITC and 5 µL PI. They were mixed and dispersed uniformly in the centrifuge tube, then incubated at 4°C in dark for 30 min, added with 150 µL Binding buffer and detected by flow cytometry within 1 h.

Effect of ALA-PDT on apoptosis of Me180 cells detected with Hoechst 33342 staining. After ALA-PDT functioned for 3 h, the medium was removed, the cells were tenderly washed with PBS twice, fixed with 4% paraformaldehyde for 15 min and washed again with PBS three times, added with 5 µg/mL Hoechst 33342 (with Ex of 346 nm and Em of 460 nm) and incubated at 37°C for 30 min, washed with PBS for 5 min three times. Finally, the slides were sealed by PBS/glycerol mixture, and were observed under the inverted fluorescence microscope.

Effect of ALA-PDT on apoptosis of Me180 cells detected by May-Grünwald-Farbstoff Giemsa method. After ALA-PDT functioned for 3 h, cytospin slides were gathered and cell climbing slides were prepared by the same way. Then the cells were fixed with methanol. The cell nuclei were stained by May-Grünwald-Farbstoff (2.5 g per 1000 mL) for 4 min. The cells were washed with distilled water. The cytoplasm was counterstained by Giemsa (0.5 g in 33 mL glycerol at 56°C for 2 h and then added with 33 mL methanol) for 15 min. Then the cells were washed with tap water. When the cells were dried, they were kept transparent by xylene and neutral resin was used to seal the slides. Cell morphology was observed under the microscope.

Effect of ALA-PDT on cell cycle distribution of Me180 cells detected by flow cytometry with PI staining. After ALA-PDT functioned for 24 h, Me180 cells were washed with PBS twice and fixed by 70% ethanol for over 24 h. After removing ethanol by centrifugation, the cells were washed with PBS twice, added with 100 µg/mL RNA enzyme and 50 µg/mL PI, incubated at 37°C for 30 min, and filtered through the 300-mesh nylon net. Cell apoptosis and cell cycle distribution were tested by flow cytometry.

Effect of ALA-PDT on RNA in Me180 cells. After ALA-PDT functioned for 3 h, total RNA was extracted from Me180 cells with QIAGEN’s RNeasy® RNA extraction kit, and RNA concentration was measured by the ultraviolet spectrophotometer. The mRNA levels of survivin, p53, bax, bcl-2, and bad were detected by reverse
transcription-polymerase chain reaction (RT-PCR). cDNA was synthesized using reverse transcriptase kit from MBI Company according to the instruction. SYBR® Premix Ex Taq™ kit from TaKaRa Company was used for fluorescence PCR. The reaction system contained 25 μL Taq™, 1 μL upstream primers (10 μmol/L) and 1 μL downstream primers (10 μmol/L), and 1 μL template DNA, and added with sterilized double distilled water to a total volume of 50 μL. The primer sequences were listed in Table 1.

The conditions of two-step PCR were as below: pre-degeneration at 95°C for 10 s; 45 cycles of reaction at 95°C for 5 s and at 60°C for 10 s. After the end of each cycle, the fluorescence intensity of each sample was detected to obtain cDNA fluorescence curves.

After PCR, the amplification of each gene was observed with automatic computer analysis. The PCR threshold value cycles were exported and were analyzed via the statistical package. β-actin was used as positive internal control and the relative quantity was calculated according to the 2-ΔΔCt method. The ΔC of target gene = the C of target gene - C of β-actin for the same sample; the ΔΔC of target gene = the ΔC of target gene in treatment group- the ΔC of target gene in control group. Finally the melting curves were monitored. After the last cycle was finished, the temperature increased to 95°C, then decreased to 60°C rapidly and maintained for 15 s, then increased to 95°C at a speed of 0.1°C/s. During the process, the fluorescent intensity was monitored continually. The melting curves were analyzed to identify whether the amplified products were single target sections.

### Table 1 - Sequences of the primers used in PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences</th>
<th>Length</th>
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<tbody>
<tr>
<td>β-actin</td>
<td>Upward, 5'-TTGTTACAGGAGTCCTTGC-3'; Downward, 5'-ATGCTATACCTTCCCTCAGTG-3'</td>
<td>101 bp</td>
</tr>
<tr>
<td>survivin</td>
<td>Upward, 5'-TCCACTCTGGGCAACTGAGAC-3'; Downward, 5'-TGCCCTCTCAGCTGCTGAT-3'</td>
<td>101 bp</td>
</tr>
<tr>
<td>p53</td>
<td>Upward, 5'-TCAAAAGATGTTTTGCCAAGT-3'; Downward, 5'-ATGCTGCTGACTGCTGATG-3'</td>
<td>77 bp</td>
</tr>
<tr>
<td>bax</td>
<td>Upward, 5'-GGGACCAGGCTCCACCCAC-3'; Downward, 5'-AGGACCAGGCTCCACCCAC-3'</td>
<td>148 bp</td>
</tr>
<tr>
<td>bcl-2</td>
<td>Upward, 5'-TCCGCATCAGGAAGGCTAGA-3'; Downward, 5'-AGGACCAGGCTCCACCCAC-3'</td>
<td>111 bp</td>
</tr>
<tr>
<td>survivin</td>
<td>Upward, 5'-TCCACTCTGGGCAACTGAGAC-3'; Downward, 5'-TGCCCTCTCAGCTGCTGAT-3'</td>
<td>101 bp</td>
</tr>
<tr>
<td>β-actin</td>
<td>Upward, 5'-TTGTTACAGGAGTCCTTGC-3'; Downward, 5'-ATGCTATACCTTCCCTCAGTG-3'</td>
<td>101 bp</td>
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</table>

The results of MTT assay showed that Me180 cell line was the most sensitive to ALA-PDT among the eight cell lines. Its IC₅₀ was around 7 mmol/L (Table 2).

### Table 2 - Effects of ALA-PDT on proliferation of 8 cervical carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>10 J/cm²</th>
<th>IC₅₀ of ALA (mmol/L)</th>
<th>20 J/cm²</th>
<th>30 J/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me180</td>
<td>(2.78 ± 0.25) × 10⁻⁴</td>
<td>(6.46 ± 0.37) × 10⁻⁴</td>
<td>(7.33 ± 0.20) × 10⁻⁴</td>
<td></td>
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<tr>
<td>C33A</td>
<td>(1.70 ± 0.13) × 10⁻³</td>
<td>(2.25 ± 0.32) × 10⁻³</td>
<td>(1.91 ± 0.53) × 10⁻³</td>
<td></td>
</tr>
<tr>
<td>HeLa</td>
<td>(1.02 ± 0.32) × 10⁻²</td>
<td>(1.11 ± 0.47) × 10⁻²</td>
<td>(1.16 ± 0.82) × 10⁻²</td>
<td></td>
</tr>
<tr>
<td>Caski</td>
<td>(3.52 ± 0.02) × 10⁻²</td>
<td>(4.01 ± 0.01) × 10⁻²</td>
<td>(4.91 ± 0.04) × 10⁻²</td>
<td></td>
</tr>
<tr>
<td>C4I</td>
<td>(7.77 ± 0.42) × 10⁻²</td>
<td>(1.00 ± 0.82) × 10⁻¹</td>
<td>(1.42 ± 0.63) × 10⁻¹</td>
<td></td>
</tr>
<tr>
<td>HT3</td>
<td>(3.32 ± 0.45) × 10⁻¹</td>
<td>(2.67 ± 0.52) × 10⁻¹</td>
<td>(7.22 ± 0.40) × 10⁻¹</td>
<td></td>
</tr>
<tr>
<td>SiHa</td>
<td>(3.22 ± 0.25) × 10⁻¹</td>
<td>(3.50 ± 0.23) × 10⁻³</td>
<td>(6.58 ± 0.42) × 10⁻¹</td>
<td></td>
</tr>
<tr>
<td>H8</td>
<td>(3.40 ± 0.10) × 10⁻¹</td>
<td>(4.90 ± 0.24) × 10⁻¹</td>
<td>(3.10 ± 0.28) × 10⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

ALA-PDT, 5-aminolevulinic acid-mediated photodynamic therapy. All values are presented as mean ± SD of 3 experiments. *p < 0.001, vs. Me180 cells.

### Results

#### Selection of sensitive cell lines. The results of MTT assay showed that Me180 cell line was the most sensitive to ALA-PDT among the eight cell lines. Its IC₅₀ was around 7 mmol/L (Table 2).

#### Proliferation inhibition of Me180 cells after treatment of ALA-PDT. The proliferation inhibition rate of Me180 cells increased along with the increase of ALA concentration from 0.0001 to 0.1 mmol/L (p < 0.01); the effect of ALA from 0.1 to 10 mmol/L stayed at a platform phase and the cell proliferation inhibition rate did not increase obviously. The effects of laser of 10, 20 and 30 J/cm² on Me180 cells were similar (p > 0.05, see Fig. 1 for details).

#### Optimal time for ALA-PDT functioning. The results of MTT assay showed that the IC₅₀ of ALA was (9.99 ± 0.24) × 10⁻³ mmol/L after ALA-PDT functioned for 1 h; it increased at 2 h and 3 h, and reached the peak of (5.05 ± 0.22) × 10⁻⁴ mmol/L at 4 h (p < 0.01); it gradually decreased at 6 h, 12 h and 24 h, and reached (1.45 ± 0.24) × 10⁻⁵ mmol/L at 24 h.

#### Effect of ALA-PDT on apoptosis of Me180 cells detected by flow cytometry with Annexin V-FITC/PI double staining. ALA-PDT apparently induced the apoptosis of Me180 cells. When functioned for 3 h, ALA-PDT mostly induced early apoptosis compared to the control group. At 24 h, when compared to those of 3 h, the early
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Effect of ALA-PDT on apoptosis of Me180 cells detected with May-Grünwald-Farbstoff Giemsa staining. ALA (1 mmol/L) combined with PDT functioning for 3 h exerted strong killing effect on Me180 cells. Using May-Grünwald-Farbstoff Giemsa staining, abundant of dead cells and apoptotic cells were observed (Fig. 3).

Effect of ALA-PDT on cell cycle of Me180 cells. After ALA-PDT functioned for 3 h, the cell cycle of Me180 cells did not change obviously. While after ALA-PDT functioned for 24 h, the cell cycle distribution of Me180 cells was changed obviously. ALA (1-2 mmol/L) combined with PDT arrested Me180 cells at G0-G1 phase; the proportions of Me180 cells at S phase and G2/M phase decreased (Table 4).

Effect of ALA-PDT on RNA in Me180 cells detected by RT-PCR. The relative mRNA levels of apoptosis-related genes, including survivin, p53, bax, bc1-2 and bad, are shown in Figure 4. The mRNA levels of survivin and bc1-2 were significantly lower in ALA-PDT group than in control group (p < 0.001), while the mRNA levels of p53, bax and bad only increased slightly after PDT treatment with no significant difference from those in control group (p > 0.05).

Effect of ALA-PDT on Survivin protein in Me180 cells detected by Western blot. ALA-PDT significantly inhibited the protein expression of Survivin in Me180 cells. The inhibition rates of Survivin expression were 48.42%, 71.72% and 73.84% respectively at 3 h after treatment of ALA with concentrations of 0.1, 1 and 2 mmol/L combined with PDT (Fig. 5).

**Discussion**

In 1990, Kennedy et al.6 firstly applied ALA-PDT in clinic. At present, ALA-PDT is widely used by local or systematic administration to treat skin cancer, esophageal cancer, lung cancer and gastrointestinal cancer. However, the application of ALA-PDT to treat cervical intraepithelial neoplasia (CIN) and early stage cervical cancer is immature.7 Compared with the research on photosensitizer, laser equipment and their clinical application, the study on the theoretic basis of PDT lags behind. Currently, the basic researches cannot describe quantitatively the relation between the photodynamic reaction and the elements for the reaction. Clinical doctors can only follow the early experience concerning PDT and treat different patients with the same protocol.8 Current researches on PDT targeting cervical cancer are insufficient. A complete theoretical system is required and the optimal conditions for PDT treatment under the guidance of the theory are needed.

Our study showed that ALA-PDT could inhibit the proliferation of eight cervical cancer cell lines. The single administration of ALA or laser radiation could not lead to obvious proliferation inhibition, which might because ALA is the precursor of the endogenous photosensitizer PpIX and has no impact on cell survival. The sensitivity

**Figure 1.** Effects of ALA-PDT on proliferation of Me180 cells

**Figure 2.** Effects of ALA-PDT on apoptosis of Me180 cells [Hoechst 33342 × 200]. (A) Only a few apoptotic cells can be seen in control group. (B) Some swelled cells can be seen after treatment of 0.1 mmol/L ALA. (C) Apoptotic cells and apoptotic bodies can be seen after treatment of 1 mmol/L ALA. (D) More apoptotic cells and apoptotic bodies, swelled cells and cell death can be seen after treatment of 2 mmol/L ALA.
of these eight cervical cancer cell lines to ALA-PDT are different, but the curves of killing effects are similar. It might be related to the types of cell lines and the concentration of PpIX in cells. Me180 is the most sensitive to ALA-PDT, and had been used in following experiments.

Our study showed that the effects of ALA-PDT on the inhibition of various cervical cancer cell lines are different, which have interlocking relation with ALA concentration and radiation dose to some extent. In Me180 cells, the cell proliferation inhibition rate increased along with the increase of ALA concentration from 0.0001 to 0.1 mmol/L; the inhibition rate stayed at a platform phase when ALA concentration increased from 0.1 to 10 mmol/L. It implies that ALA at a relatively high concentration leads to protoporphyrin saturation. No significant difference was found in the effects of laser of various doses (10, 20, and 30 J/cm²) on the apoptosis of cervical cancer cells. We hypothesized that PDT of 10 J/cm² is enough for the killing effect of ALA-PDT on cervical cancer cells. The combination of relatively low ALA concentration and relatively high laser dose will guarantee the efficacy and avoid adverse effects. When ALA concentration increases to a certain level, the amount of protoporphyrin reaches saturation, cell proliferation inhibition rate does not increase significantly; if continue to increase ALA concentration, adverse effects increase. When laser dose is too low, the therapy effect cannot be achieved; if the laser dose increases too high, injuries to normal tissues appear. Literature reported different optimal function parameters for PDT, which is thought to be related to different cell types, photosensitizers, wavelengths, and other factors.

In our study, the proliferation inhibition of Me180 cells appeared when treated with ALA for 1 h, enhanced gradually at 2 and 3 h, reached the peak at 4 h, and decreased gradually at 6, 12 h and 24 h. It is thought that cell proliferation partially compromises the killing effect of ALA-PDT. Our findings showed that 2 mmol/L ALA, laser of 10 J/cm², and 3-hour incubation are optimal conditions for killing Me180 cells.

The present study found that ALA-PDT could significantly induce the apoptosis of Me180 cells. After ALA-PDT functioned for 3 h, the total apoptosis rate of Me180 cells significantly increased by 52.45% (1 mmol/L ALA) and 77.92% (2 mmol/L ALA) compared to that of control group. After ALA-PDT functioned for 24 h, the early apoptosis rate and the total apoptosis rate of Me180 cells decreased compared to that of 3 h, which may due to the time phases. ALA (1 and 2 mmol/L) combining PDT arrested Me180 cells at G₀/G₁ phase, inhibited cell proliferation. The result is similar to that reported by Haywood-Small et al. Inducing the apoptosis of Me180 cells is the main mechanism of ALA-PDT.

Table 3. Effects of ALA-PDT on apoptosis of Me180 cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Early apoptosis rate (%)</th>
<th>Late apoptosis rate (%)</th>
<th>Total apoptosis rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 h 24 h</td>
<td>3 h 24 h</td>
<td>3 h 24 h</td>
</tr>
<tr>
<td>Control</td>
<td>0.62 ± 0.11 0.86 ± 0.15</td>
<td>0.65 ± 0.08 0.76 ± 0.11</td>
<td>1.31 ± 0.30 1.61 ± 0.16</td>
</tr>
<tr>
<td>ALA-PDT</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.1 mmol/L</td>
<td>8.21 ± 1.24a 3.67 ± 1.24b</td>
<td>5.23 ± 0.85a 3.26 ± 0.98b</td>
<td>13.42 ± 1.56a 6.92 ± 1.18b</td>
</tr>
<tr>
<td>1 mmol/L</td>
<td>33.40 ± 5.63a 14.84 ± 4.30a</td>
<td>19.11 ± 3.42a 16.63 ± 2.31a</td>
<td>52.45 ± 5.48a 31.51 ± 4.76a</td>
</tr>
<tr>
<td>2 mmol/L</td>
<td>54.89 ± 5.34a 22.54 ± 3.23a</td>
<td>23.15 ± 4.45a 15.22 ± 2.67a</td>
<td>77.92 ± 5.23a 37.65 ± 3.09a</td>
</tr>
</tbody>
</table>

All values are presented as mean ± SD of three experiments. *p < 0.001, **p < 0.01, vs. control.
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Different photosensitizers and different doses of laser function on different cells through different cellular signaling pathways to induce apoptosis or death, which seems intricate.\(^\text{12}\) PDT induces apoptosis mainly through two approaches: death receptor-mediated apoptosis and mitochondria-mediated apoptosis. The approach of mitochondria-mediated apoptosis has been verified by some researches, while the approach of death receptor-mediated apoptosis needs to be further researched.\(^\text{13}\) Our results showed that ALA-PDT could significantly inhibit the mRNA and protein expression of survivin, and the mRNA expression of bc1-2 in Me180 cells. Cell death resulting from PDT is thought to relate to survivin and bc1-2. Mitochondria-related Caspase-3 and Caspase-9 pathway may be the main pathway for PDT to induce cell apoptosis. It implies that controlling or changing apoptosis-related genes for PDT-insensitive cells can further improve the efficacy of PDT on cancers.

References


