# 5-氨基乙酰丙酸介导的光动力学治疗胃癌的实验研究

Gastric cancer is the second leading cause of cancer-related deaths worldwide[1], whose treatment depends largely on radical resection of the primary tumor. As a new clinical treatment modality for cancer in addition to chemotherapy and radiotherapy, photodynamic therapy (PDT) is based on preferential accumulation of photosensitizers in the tumor, which, when absorbing light of appropriate wavelengths, produce cytotoxic oxygen product to cause direct cell death and/or vascular shutdown[2][3][4].

5-aminolaevulinic acid (5-ALA) is an endogenous substrate in the haem biosynthetic pathway. Protoporphyrin IX (Pp IX), the immediate haem precursor in the pathway, has photoexcitable properties[5]. Because of the significant difference in the activities of key enzymes in the heme pathway between tumor and normal tissue, PpIX accumulation induced by ALA in tumor cells is higher than that in normal cells. ALA-PDT therefore has good tumor selectivity, and with the short half-life of 5-ALA, this treatment may reduce skin photosensitivity to 1 or 2 days from 1 or 2 months with other traditional photosensitizers [6][7].

In this study, the authors aimed to investigate the response of human gastric adenocarcinoma MGC-803 cell line to ALA-mediated PDT in vitro.

## MATERIALS AND METHODS

### Agent preparation

5- ALA obtained from Sigma (St. Louis, MO) was dissolved in PBS (pH 7.0) and the stock solution of 24 mmol/L was kept at 4 ℃ before use.

### Cell culture

Human gastric cancer (MGC-803) cells supplied by the Experimental Animal Center of Sun Yat-sen University were routinely cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, Gibco BRL) at 37 ℃ in a humidified incubator containing 95% air and 5% $CO_2$. The cells in exponential growth phase were used in the experiments.

### Cell viability assay

PDT-induced phototoxicity of MGC-803 cells was determined by means of MTT assay (Sigma, St. Louis, MO)[8] and the cell survival rate was calculated according to the formula: Cell survival rate (%) = (OD value of treated cells/OD value of control cells)× 100%.

Photodynamic treatment

Effect of PDT with different 5-ALA concentrations

MGC-803 cells washed with fresh serum-free medium for 3 times were plated into 96-well flat-bottomed culture plates at 5×104 cells per well. After cell attachment to the substratum, the cells in PDT groups were treated with 5-ALA at different concentrations (0.25, 0.5, 1.0, 2.0, 4.0 mmol/L, respectively) in serum-free medium, and incubated for 6 h. The cells of both PDT and control groups were subsequently exposed to irradiation with 635 nm laser from a diode laser generator (630PDT Laser, Diomed, UK) at 25.0 J/cm$^2$[8][9]. After the exposure the cells were incubated with fresh medium containing 10% FBS for 24 h and the cell viability was determined by MTT assay.

Effect of PDT with different laser doses

The cells were treated as described above but 5-ALA concentration in each PDT group was 1.0 mmol/L[8][9], and cells were subjected to laser exposure at 6.25, 12.5, 25.0, 50.0, 100 J/cm$^2$; respectively.

Effect of different 5-ALA concentrations on MGC-803 cells

The cells were treated as described above, and incubated with 5-ALA of 0.25, 0.5, 1.0, 2.0, 4.0 mmol/L, respectively without laser irradiation.

Effect of different laser doses on MGC-803 cells

The cells treated as described above were irradiated with laser at the doses of 6.25, 12.5, 25.0, 50.0, and 100 J/cm$^2$, respectively, but without incubation with 5-ALA.

Data analysis and statistics

The data were analyzed with SPSS 10.0 software and the original data were presented as Mean±SD. ANOVA was performed for statistical analysis. A P value less than 0.05 was considered to indicate statistical significance.

## RESULTS

After laser exposure of 25.0 J/cm$^2$ and incubation with different concentrations of 5-ALA, the survival rates of MGC-803 cells were (70.07±5.37)%, (50.04±4.99)%, (34.53±5.30)%, (26.89±4.44)%, and (23.90±2.80)% for 5-ALA concentration of 0.25, 0.5, 1.0, 2.0, and 4.0 mmol/L, respectively, showing significantly different cytotoxicity of 5-ALA ($F=266.39$, $P<0.001$).

After incubation with 1 mmol/L 5-ALA and exposure to different laser doses, the survival rates of MGC-803 cells were (83.50±10.43)%, (67.96±9.23)%, (33.80±8.26)%, (23.31±5.98)%, (14.96±3.50)% for laser doses of 6.25, 12.5, 25.0, 50.0, and 100 J/cm$^2$, respectively. The survival rate was significantly decreased with increment of the laser dose ($F=226.31$, $P<0.0001$).

The survival rates of MGC-803 cells incubated with different concentrations of 5-ALA but without laser exposure were (96.46±6.72)%, (97.48±5.27)%, (98.33±6.67)%, (99.47±6.97)%, and (95.66±7.72)% for 5-ALA concentrations of 0.25, 0.5, 1.0, 2.0, and 4.0 mmol/L, respectively, showing no significant difference in the cytotoxicity ($F=0.79$, $P=0.5383$)

Without 5-ALA incubation, laser exposure at different doses did not produce significant difference in the survival rates of the cells, which were (98.85±6.09)%,

(96.70±7.12)%, (97.49±6.07)%, (95.70±5.08)%, and (98.11±6.33)% for laser doses of 6.25, 12.5, 25.0, 50.0, and 100 J/cm$^2$, respectively (F=0.61, P=0.6551).

## DISCUSSION

In this study we found that laser exposure of MGC- 803 cells at the same dose resulted in significantly lowered cell survival rate from (70.07±5.37)% to (23.90±2.80)% (P<0.0001) in proportion to the 5-ALA concentration increment from 0.25 to 4.0 mmol/L (P<0.01). After treatment with 5-ALA ranging from 0.25 to 2.0 mmol/L, the cell survival rate decreased sharply whereas within the concentration range of 2.0 to 4.0 mmol/L, 5-ALA resulted in only less obvious reduction in the cell survival rate (P>0.05). In ALA-PDT, 5-ALA itself does not serve as a photosensitizer but as the biological precursor in the heme biosynthetic pathway[5][6][7], which produces protoporphyrin IX (Pp IX), a potent photosensitizer, in response to 5-ALA-induced endogenous photosensitization. Cellular PpIX production increased, as suggested by our findings, after treatment of the cells with 5-ALA at relatively lower concentrations, and this increment exhibited saturation with higher concentrations of 5-ALA. As PpIX is the product in the heme biosynthetic pathway whose biosynthetic capacity is limited, the saturation of cellular PpIX production at higher 5-ALA concentration does not seem to be surprising. This finding may potentially help in deciding the optimal dose of 5-ALA in future clinical PDT.

In another section of the experiment in which MGC-803 cells were incubated with the same dose of ALA before laser exposure at different doses, the cell survival rate was found to significantly decrease with the increment of the laser doses. This is due to increased energy absorption by the photosensitizers that generate increasing active oxygen species, hence greater cell killing effect. However, other factors such as self-shielding and photobleaching (self-destruction of the photosensitizer during the PDT) may complicate precise laser dose delivery[10], so that excessive laser dose in PDT may not produce photocytotoxicity of the cells in proportion to the laser dose; on the other hand, desired photochemical effect cannot occur with excessively low laser dose. In this experiment, neither of the two conditions occurred, indicating that the laser doses we chose were appropriate.

The survival rates of the MGC-803 cells incubated with different concentrations of 5-ALA without laser exposure did not differ significantly (F=0.7853, P=0.5383), suggesting that 5-ALA by itself cannot be activated to produce cytotoxicity, as 5-ALA only serves as the non-toxic precursor of Pp IX, the actual photosensitizer.

The survival rates of the cells with laser exposure at various doses but without 5-ALA incubation also showed no significant difference (F=0.6122, P=0.6551), which further justified the appropriateness of the laser dose we chose.

In conclusion, the results of this study demonstrate that 5-ALA itself is nontoxic, and 5-ALA-mediated PDT can be a promising method for clinical management of gastric cancer.

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