Porphyrin Derivatives Mediated Sonodynamic Therapy on Malignant Glioma Cells in Vitro

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This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 26670632.
Sonodynamic therapy (SDT)

- Sonosensitizer
- Ultrasound (US)
- Cavitation phenomenon
- Microstreaming
- Sonochemical reaction

Singlet state → Ground state

*Reactive Oxygen Species (ROS): singlet oxygens, hydroxyl radicals, superoxide anions, hydrogen peroxides

Introduction

Sonodynamic therapy using water-dispersed TiO$_2$-polyethylene glycol compound on glioma cells: Comparison of cytotoxic mechanism with photodynamic therapy
**Methods**

**SDT protocol**

- **Cell plating**: $3.0 \times 10^5$ cells/well
  - C6
  - U87MG

- **Sonosensitizer co-incubation**
  - ALA concentration: 200 $\mu$g/ml
  - PpIX concentration: 1.0 $\mu$g/mL
  - TS concentration: 30 $\mu$g/mL

- **US exposure**
  - Frequency: 1.0 MHz
  - Duty ratio: 50%
  - $I_{sata}$: 0.16 W/cm$^2$
  - Duration: 60 seconds

- **Cell viability**
  - Calcein AM / EthD-1
  - Hoechst33342

**US apparatus (scheme)**
- Transducer
- Water bath (keep at 37°C)
- Gas removal equipment
- Water temperature gauge
- Oscilloscope (DPO2012, Tektronix, Tokyo, Japan)
- Amplifier (UOD-WB-1000, Tokin, Miyagi, Japan)
- Function generator (AFG3022, Tektronix, Tokyo, Japan)

**Cell damage mechanism**
- Annexin V-FLUOS
- PI

<table>
<thead>
<tr>
<th>Function generator (mV)</th>
<th>350</th>
<th>495</th>
<th>606</th>
<th>700</th>
<th>778</th>
<th>850</th>
<th>985</th>
<th>1100</th>
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<tbody>
<tr>
<td>$I_{sata}$</td>
<td>0.08</td>
<td>0.16</td>
<td>0.24</td>
<td>0.32</td>
<td>0.40</td>
<td>0.48</td>
<td>0.64</td>
<td>0.8</td>
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</table>
① Average number of EthD-1 (+) and Hoechst33342 (+) counted cells at 5 points in each well was calculated in each group (Control, ALA alone, US alone, SDT group; n=3).

② Exfoliated cells (Presumptive dead cells) = Control Hoechst33342 (+) counted cells − Treated Hoechst33342 (+) counted cells

③ Compensated dead cells = EthD-1 (+) counted cells + ②

④ Survival rate (%) = 100 − ③ / Control Hoechst33342 (+) cells × 100
**Results 1:** Comparison of the cytotoxicity of SDT between C6 and U87MG cells

* : p<0.05  By one-way ANOVA followed by Tukey-Kramer test
Results 2: Fluorescence intensity per cell after co-incubation with each sonosensitizer

 excitation 488 nm, emission 690/50 nm
Results 3: *Cell damage mechanism of ALA-SDT on C6 cells* (at 0.16 W/cm², 60 sec)

- **Control**
- **ALA alone**
- **US alone**
- **SDT**

Live cells    Early apoptotic cells

Late apoptotic cells    Necrotic cells

Excitation 488 nm, emission 530/30 nm (Annexin V) and 574/26 nm (PI)
The efficacy of SDT has been shown to closely correlate with the concentration of sonosensitizer in target tumor cells.

Yumita N, Cancer Chemother Pharmacol, 2010

The efficacy of SDT was improved by inhibition of the function of ABCG2 transporter, which regulates cellular accumulation of porphyrin derivatives, with specific inhibitor in the glioma stem-like cells isolated from human glioblastoma cell line U251MG.

Xu ZY, Ultrasonics, 2013

Discussion: Comparison of the cytotoxicity of SDT among different cell lines
• Our study found that the cytotoxicity of sonication of glioma cells is enhanced by each sonosensitizer and that the efficacy of sonodynamic therapy may depend on the degree of intracellular accumulation of sonosensitizer.

• Induction of apoptosis was suggested to be a major mechanism underlying cell death.

• SDT with porphyrin derivatives (ALA, PpIX and TS) might be a novel feasible therapeutic modality for glioma.