Photodynamic Anti-Tumor Efficiency of Hematoporphyrin Derivative

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ABSTRACT

The photophysical property, cytotoxicity of hematoporphyrin derivative BL-1 in vitro and in vivo were investigated as photosensitizer for photodynamic therapy (PDT). Strong absorption and high singlet oxygen quantum yield of compound BL-1 were exhibited. Significant phototoxicity and inhibition to tumor were also showed. So compound BL-1 was suggested as the potential and promising antitumor photosensitizer for PDT.

Keywords: Photosensitizer, Photodynamic therapy, Hematoporphyrin derivative, Tumor

Abbreviations: PDT: Photodynamic Therapy; PS: Photosensitizer; HPD: Hematoporphyrin Derivative; HMME: Hematoporphyrin Monomethyl Ether

Short Communication

Photodynamic therapy (PDT) is an emerging non-invasive treatment based on a combination of photochemistry and photophysics. PDT has more advantages compared with traditional chemotherapy and surgical treatment, which is more targeted, less harmful to healthy tissue [1-4]. When the photosensitizer (PS) molecule is illuminated by a light of particular wavelength, it passes from the first excited singlet state to the first excited triplet state T1, which will initiate a photochemical reaction by generating reactive free radicals or transferring its energy to the ground state oxygen molecule (3O2) to produce singlet oxygen (1O2) that can oxidate the bio-molecules of diseased cells and apoptosis or necrosis was resulted [5,6]. Photodynamic therapy was discovered nearly 100 years ago but medically approved drugs and technologies are still limited [7,8].

The first photosensitizer used in clinics was Hematoporphyrin Derivative (HPD) which was called Photofrin or porfimer sodium. It is a mixture of six hematoporphyrin derivatives with long retention in skin which resulted in that patient need to avoid the light for 4 weeks. Hemeporfin (HMME) is a mixture of two enantiomers which was first discovered by Xu [9]. HMME has better performance than porfimer sodium. It shows low toxicity to normal tissues with rapid metabolism property. For nearly a century, the design and synthesis of hematoporphyrin derivatives are still of concern to many scientists. Here the photophysical property, cytotoxicity of new hematoporphyrin derivative BL-1 in vitro and in vivo were investigated As Photosensitizer for Photodynamic Therapy (PDT).

Results and Discussion

Photophysical Properties

Compound BL-1 was dissolved in DMSO. UV-vis spectrum and fluorescence emission spectrum of Compound BL-1 were investigated. As shown in (Figure 1) BL-1 had the characteristic soret and Q band absorptions at 401nm (soret), 498nm, 532nm, 569nm and 622nm (Q band), respectively. Compound BL-1 displayed a fluorescence excitation band at approximately 400nm and emission band at 620nm. HMME was chosen as the control compound.
Singlet Oxygen

The efficiency of singlet oxygen can be used to evaluate the cytotoxicity of the photosensitizer. The singlet oxygen generation of the photosensitizer is detected by DPBF chemical oxidation method for the singlet oxygen capacity of the photosensitizer. The slope of the function is obtained from a plot of \( \ln \left[ \frac{[\text{DPBF}_0]}{[\text{DPBF}_t]} \right] \) versus illumination time \( t \). As shown in (Figure 2), the absorption intensity of DPBF (\( \lambda = 410\text{nm} \)) continuously decreased in the presence of BL-1 as the irradiation time increasing. The rate of singlet oxygen generation was 0.0022 min\(^{-1}\).

MTT Assay

High phototoxicity and low dark toxicity are important factors for evaluating the effectiveness of photosensitizers. The MTT assay was used to evaluate the phototoxicity and dark toxicity of the compounds BL-1. The control group was set with different concentrations of DMEM in DMSO. The data demonstrated that DMSO had no effect on the survival rate of experimental cells (data not shown). At the same dose of light, drug concentration was inversely correlated with cell viability. At the same concentration of PS, the light dose was also negatively correlated with cell viability.

The IC50 (the concentration of a photosensitizer inhibits 50% of the cells under light) values were shown in (Table 1). The IC50 value of BL-1 to Eca-109 tumor cells was smaller than HMME. These results suggested that BL-1 was a promising photosensitizer in vitro.
Tumor Inhibition Rate

The inhibition rate of phototoxicity for BL-1 toward Eca-109 was calculated as follows:

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL-1</td>
<td>6.03*</td>
</tr>
<tr>
<td>HMME</td>
<td>9.46</td>
</tr>
</tbody>
</table>

Note: Visible light 622 nm, light dose 16 J/cm².

*P < 0.05 represents a significant difference relative to the control group.

Table 1: Phototoxicity for BL-1 and HMME toward Eca-109 cells.

Photodynamic Activity In Vivo

PDT preferentially damages photosensitizer target cells. This statement can be explained by the current research about HIF mechanism. When the tumor site is hypoxic, HIF is highly expressed. This process promotes the growth of blood vessels and red blood cells at the tumor site. Porphyrins are coenzymes of red blood cells. Therefore, it will preferentially accumulate in the tumor site, and has a certain tumor targeting effect [10-12]. The antitumor activity of compound BL-1 was evaluated on Balb/c nude mice bearing Eca-109 tumor. Tumor inhibition rates were measured by calculating the weight of nude mice tumors. The experiment was divided into 4 groups containing 4 mice per group: control group, light group, PS group and PDT group. The tumor weight of the nude mice gradually increased in the control group, the light group and the photosensitizer group. The weight of tumors is gradually decreasing as the photodynamic therapy group grows with time. Tumor inhibition rate is shown in (Table 2). It is suggested that the compound BL-1 exhibited efficient photodynamic antitumor efficacy on Balb/c nude mice at lower concentration. Therefore, BL-1 is a promising antitumor PS in PDT application.

Table 2: The inhibition rate of phototoxicity for BL-1 toward tumor inhibition rate.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor Inhibition Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>--</td>
</tr>
<tr>
<td>light</td>
<td>2.05±0.62*</td>
</tr>
<tr>
<td>PS</td>
<td>5.32±0.59*</td>
</tr>
<tr>
<td>PS-PDT</td>
<td>85.32±2.13***</td>
</tr>
</tbody>
</table>

Note: *P < 0.05, ***P < 0.001 represents a significant difference relative to the control group.

Cell Lines and Culture Conditions

The cells were obtained from the Cell Culture Center of the Chinese Academy of Sciences. The cell-related reagents were purchased from Shanghai Mingrong Biotechnology Co., Ltd. The cells were cultured in 10% Fetal Bovine Serum (FBS), 50mg/mL penicillin and 50mg/mL. In medium culture, 5% carbon dioxide was added to a humidified incubator at 37°c. Cells in the exponential growth phase were used in each experiment.

MTT Assay

The experiment of cell phototoxicity and dark toxicity was performed using the MTT assay. Cells of various concentrations were cultured in DMEM medium containing 10% (v/v) FBS and allowed to ingest for 24 hours in the dark. Cell viability was assessed by 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-bromide triazole (MTT) colorimetric assay 24 hours after treatment.

In Vivo Experiments

When the size of the tumor reached about 100 mm3 (about 14 days after inoculation), the tumor-bearing mice were divided into 4 groups containing 4 mice per group: control group, light (without drug), PS (without light) and PS-PDT (with drug and light). After PDT, tumor regeneration or healing of the mice was observed daily.

Conclusion

The photophysical and photochemical properties of new hematoporphyrin derivative BL-1 were determined. In vivo and in vitro tests were performed to evaluate the ability of compound BL-1 to destroy tumor. The compound exhibited long maxima absorption wavelengths and higher phototoxicity compared to HMME. BL-1 exhibited better inhibitory effect on Eca-109 tumor cells than HMME. Therefore, compound BL-1 could be a potential anti-tumor photosensitizer in photodynamic therapy.

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