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PHOTOSENSITIZING EFFECTS OF A NEW 5-AMINOLAEVULINIC ACID DERIVATIVE FOR PHOTODYNAMIC THERAPY IN VITRO AND IN VIVO

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Photodynamic therapy (PDT) was a promising noninvasive treatment, which had been used in clinics for the treatment of localized tumors. The antitumor effects of a new 5-aminolaevulinic acid derivative (N-*tert*-butoxycarbonyl-[N'-benzyloxycarbonyl-(2'-oxo)-butyl] Glyciamide, BBOG) on SMMC - 7721 cells in vitro and S180 tumor model in mice were evaluated in the present paper. BBOG could induce the synthesis of protoporphyrin IX (PpIX) through SMMC - 7721 incubating. The accumulation of PpIX in SMMC - 7721 cells was measured by UV-Vis spectrophotometer and Fluorescence spectrometer. Cell viability was analyzed by MTT assay. S180 tumor model was used to evaluate the antitumor effects of BBOG - mediated PDT. Maximum peaks of UV-Vis absorption spectrum and emission spectrum were 405 nm and 643 nm, which were the characteristic peaks of PpIX respectively after incubation of SMMC - 7721 with the existence of BBOG. It could be suggested that BBOG could be bio-transferred into PpIX. MTT assay showed that the effects of BBOG - PDT had a dose - dependent relationships with drug concentration and light dosage. In mice bearing osteosarcoma S180 tumors, superior anti-tumor effect was showed among the combined use of 100 mg/kg BBOG and 180 J/cm ^{2}. It was found that BBOG had excellent anti-tumor effect in vitro and in vivo, which could be selected as a potential photosensitizer of PDT in tumor treatment.

Keywords: Photodynamic therapy; Photosensitizer; 5-aminolaevulinic acid derivatives; Tumor

INTRODUCTION

Photodynamic therapy (PDT) is a nonthermal technique for inducing tumor damage with light following administration of a light-activated photosensitizing drug. A promising approach in PDT involves the exogenous administration of 5-aminolaevulinic acid (ALA), which is a naturally occurring compound present in mammalian cells that can be metabolized to a porphyrin photosensitizer, protoporphyrin IX (PpIX), via the heme biosynthetic pathway.(Bourre *et al*., 2008) Clinically, when sufficient intracellular levels of PpIX are attained, the targeted tissue is irradiated with visible light to activate the sensitizer and trigger a chain of events that ultimately result in cell death(Giuntini *et al*., 2009). The principal advantages of ALA - PDT are the short duration of skin photosensitivity and its efficacy using both topical and oral administration.A significant drawback of ALA - PDT is the fact that ALA is a zwitterion at physiological pH resulting in low lipid solubility and limiting passage through biological barriers such as cellular membranes. To overcome this problem, several chemical approaches have been attempted to improve the incorporation of ALA and also its selectivity (Bourre *et al*., 2009).So 5-Aminolaevulinic acid ester derivatives have been widely studied. Herein, the photobiological studies of a new 5-aminolaevulinic acid derivatives are reported in vitro and in vivo. The photophysical and photochemical properties also have been evaluated.

MATERIALS AND METHODS

Chemicals

BBOG was synthesized by our independent laboratory, dimethyl sulfoxide (DMSO) was obtained from

Sinopharm Chemical Reagent Co., Ltd. All the chemicals and reagents were of analytical grade and used without any purification.

Cells

SMMC - 7721 cells were obtained from the type culture collection of the Chinese Academy of Science and cultured in normal RPMI - 1640 culture medium with 10 % fetal bovine serum (FBS), 50 units/ml penicillin, 50 µg/ml streptomycin. All cell culture related reagents were purchased from shanghai Mai Ye Bio-science Technology Co., Ltd. Cells were maintained in 5 % CO_2, 21 % O_2 at 37 °C.

Absorption and emission spectra

UV-Vis absorption spectrum was recorded on an ultraviolet visible spectrophotometer (Model V - 530, Japan). Fluorescence spectra were measured on a Fluorescence spectrometer (FluoroMax - 4, France). Slits were kept narrow to 1 nm in excitation and 1 nm in emission. Right angle detection was used. All the measurements were taken at room temperature in quartz cuvettes with path length of 1 cm.

BBOG could induce the synthesis of PpIX through SMMC - 7721 incubating 5 h in 6-well plates (Berger *et al*., 2000), the sample were PpIX and DMSO.

MTT cell viability assay

Cell viability was assessed using the 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric assay. Medium containing BBOG (0.5mM,1mM, 2mM) was administered to cells and allowed to uptake for 5 hr, than medium containing BBOG was removed and cells washed in PBS before irradiation with 2J/cm^2 , 4J/cm^2, 8J/cm^2 light (fluence rate 40 mW/cm^2) using an Nd: YAG laser at 635 nm. MTT solution was added 24 hr following PDT with absorbance of the DMSO - solubilized formazan crystals measured at 570 nm using an ELISA plate reader at 570 nm (Bio-Rad, CA, USA)(Zhang *et al*., 2014b).

Animal models

5×10^6 S180 cells were injected subcutaneously in 200 µL PBS into right abdomen on five-week-old Kunming mice. The tumors were allowed to grow to an approximate diameter of 5 - 7 mm.

In vivo photodynamic therapy

BBOG were injected to the mice bearing S180 tumors via the lateral tail vein at a dose of 100 mg/kg in 0.2 mL solution. PDT was performed at 3 h following injection with laser light (635 nm (Casas *et al*., 1999), 180 J/cm^2, 150 mW/cm^2). Visible tumors were measured using two orthogonal measurements L and W (perpendicular to L), and the volumes were calculated using the formula $V = LW^2/2$ and recorded.

Statistical analysis

All experiments were performed in triplicate, and the data were expressed as mean plus and minus the standard error of the mean. Analysis of variance (ANOVA) and Student's t test were used to determine the statistically significant difference among different groups when appropriate.

The experiments were carried out in accordance with the guidelines issued by the Ethical Committee of Donghua University.

RESULTS

UV–Vis absorption and fluorescence spectra

BBOG could induce the synthesis of protoporphyrin IX (PpIX) in SMMC -7721 after incubating 5 h in 6-well plates

(Berger *et al*., 2000), the absorption peak of PpIX in DMSO was at 405 nm (**Fig 1a**). Fluorescence spectra were measured using spectrofluorimeter described in section experimental procedures. PpIX could be excited at 405 nm, and its emission spectrum was monitored at wavelengths 643 nm (**Fig 1b**). 405 nm and 643 nm were PpIX's characteristic peaks, meant the existence of PpIX.

MTT cell viability

The effect of different concentration of BBOG on SMMC - 7721 cells was evaluated in the dark and upon exposure to different light doses by the MTT assay (**Fig 2**). No dark cytotoxicity was observed when cells exposed up to 2 mM BBOG. After irradiation, BBOG was found to be cytotoxic, with concentration and light doses increasing, SMMC-7721 cells viability reduced, BBOG-PDT increased.

In vivo photodynamic therapy

S180 tumor model was used to evaluate the anti-tumor effects of BBOG-mediated PDT. BBOG (100 mg/kg) and DMA: PEG 400 (2:3) in the same volume were injected into tumor-bearing mice via tail vein. 3h later, tumor sites of all mice were irradiated by the 635 nm laser for 20 min.No apparent side effects were observed in PDT-treated mice. After treatment 1 day, the S180 tumors became necrotic and dark, ulcers increased by 3 days after treatment, hardened and dried over the course of the next 4 days, 2 weeks later, trauma began to heal and new skin grew up, eventually formed a scab, when 21 days, the mice cured. In contrast, the tumors in control continued to grow and were significantly larger than in PDT-treatment (**Fig 3**).**Fig 4** showed the tumor growth rate curve of different treatment groups. The tumors (control group, photosensitizer group, light group) continued to grow and were larger than in BBOG-PDT-treated group (photosensitizer + light) after 5 days post-treatment, which indicates the effect of the therapy.

DISCUSSION

In this paper, we show how photodynamic treatments affecting cultured SMMC - 7721 cells and S180 tumors with new 5-aminolaevulinic acid derivatives BBOG. Both in vitro and in vivo, tests suggests that BBOG has the potential to be a photosensitizer for PDT.

One disadvantage of the currently approved PDT photosensitizers is their low absorbance in the optical window for photosensitizer excitation. In the visible spectral regions below 600 nm, light penetration into the skin is only a few millimeters which reduce the efficiency of PDT(Zhang *et al*., 2014a). **Fig 1b** shows that PpIX with >600 nm emission wavelengths allows access into deeper biological tissues for effective treatment of abnormal tissues of larger volume.

BBOG induces no dark toxicity in the range of concentrations used in the present photodynamic studies in vitro. Cell survival after incubation with BBOG and red light irradiation is related to both the BBOG concentration and light dose (**Fig 2**). It shows significant concentration and light dose dependence.

S180 is a type of very malignant tumor cell line. After treatment, even though a few cells remain, they are always expanding and proliferating, which makes it difficult to cure. **Fig 3** shows that there is a significant difference between the therapeutic group and the control group in vivo, the S180 tumors in control continue to grow and are significantly larger than in PDT-treated post-treatment(Zhang *et al*., 2015), and tumors in PDT treatment are cured at last. It proves that BBOG is an excellent photosensitizer for PDT.

UV–Vis absorption and fluorescence spectra

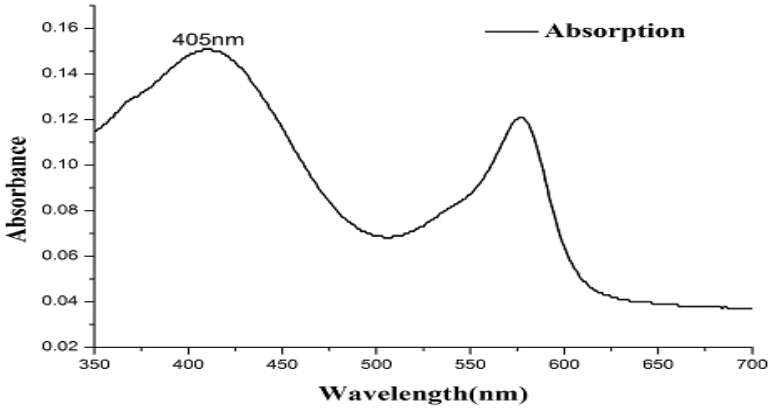

Fig 1a. UV-Vis absorption spectrum of PpIX through SMMC - 7721 incubating in DMSO, and its maximum absorbance was at 405 nm..

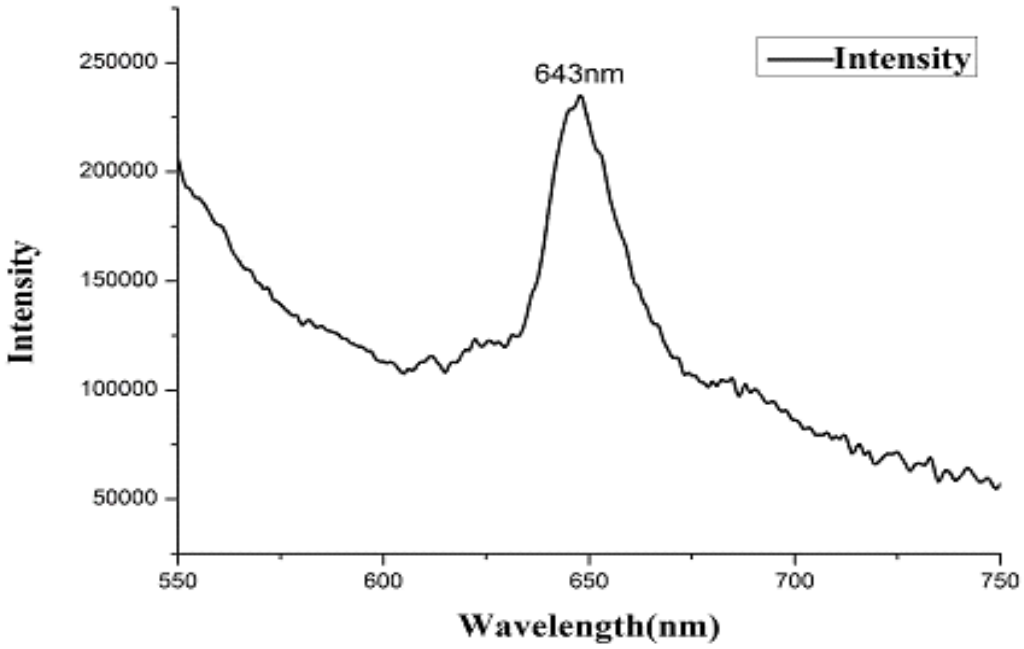

Fig 1b. Emission spectrum of PpIX after incubation of SMMC - 7721 and BBOG in DMSO, which was excited at 405 nm, and its peaks of emission spectrum were at 643 nm.

MTT cell viability

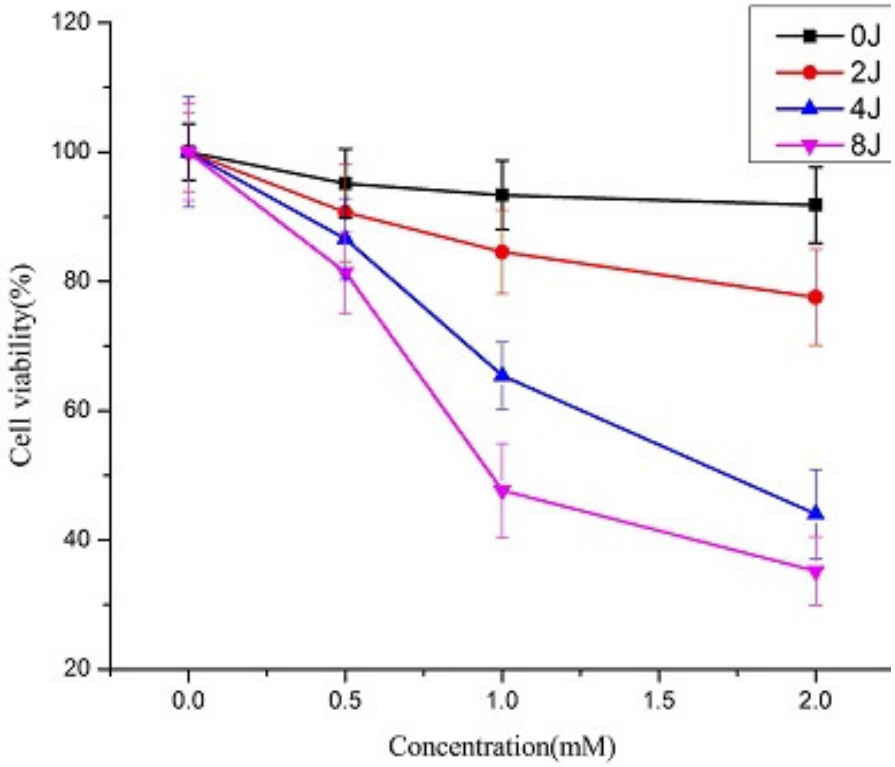

Fig 2. Light dose - effect curves for BBOG in SMMC - 7721 cells. SMMC - 7721 cells were exposed to doses from 0.5 to 2 mM for 5 h; then, cells were irradiated at a fluence rate of 40 mW/cm^2 and total light doses ranging from 2 to 8 J/cm^2. Cell cytotoxicity was determined 24 h after the end of irradiation by MTT test. The data shown are the mean ± SD of three independent experiments.

In vivo photodynamic therapy

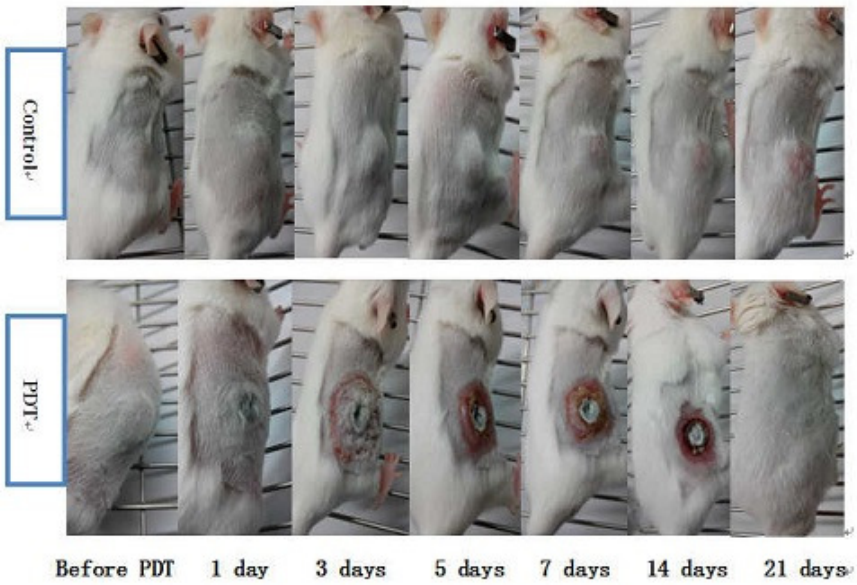

Fig 3. Images of mice bearing S180 tumors before and following PDT at each time point. Mice with saline were set as control.

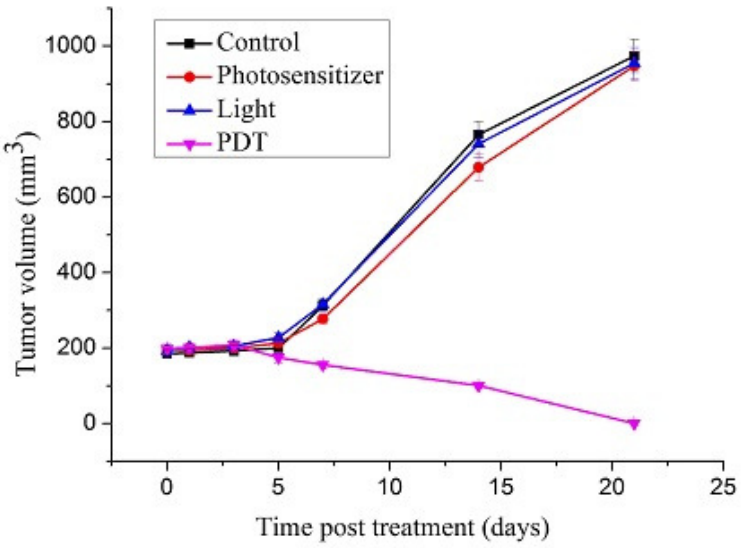

Fig 4. Tumor growth rate curve at different time points among different treatment groups. The data shown was the mean ± SD of three independent experiments.

CONCLUSIONS

BBOG displays many properties can to be considered as a good photosensitizer for PDT. It is endowed with high absorption coefficients in red spectral region at 643 nm, and is able to inactivate SMMC - 7721 cells with high efficiency in vitro. In vivo, when exposed to 635 nm laser light irradiation, S180 tumors in mice are destroyed. The above results reveal that BBOG has the potential in PDT application.

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