Journal of Photoscience (2002), Vol. 9(2), pp. 512–514

The Effects of ALA-PDT on Leukemia Cells and Hepatoma Cells

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5-aminolevulinic acid (ALA) is a new kind drug used in photodynamic therapy. ALA-PDT have successfully used in superficial malignancies and some skin diseases. Here the effects of ALA-PDT were studied on leukemia cells and hepatoma cells to explore the application on different kind cancers. It was found from the fluorescence emission spectra, that after ALA incubation the sensitizer – protoporphyrin IX (PpIX) was endogenously produced in both leukemia and hepatoma cells. The fluorescence images showed that the PpIX distribute in cytoplasm. However the efficiency of ALA photodynamic inactivation to two cell lines was different. The leukemia cells were more sensitive for ALA-PDT than hepatoma cells, revealing that the ALA-PDT effect is cell line dependent. However by using ALA-Hexyl ester (He-ALA) instead of ALA, the cell photo-inactivation was improved. The PDT efficiency of He-ALA was 10 times high than that of ALA, showing He-ALA is a very promising drug in ALA-PDT.

Key words: 5-aminolevulinic acid (ALA), leukemia cells, hepatoma cells

INTRODUCTION

5-aminolevulinic acid (ALA), a precursor of haemoglobin in heme biosynthetic pathway, was used to stimulate endogenous protoporphyrin IX (PpIX) production in tumor [1]. PpIX is an effective sensitizer, when being irradiated with suitable wavelength light it will initiate photosensitization to destroy the cancer cells. This approach was developed rapidly and has become a new branch in the field of photodynamic therapy (PDT), called ALA-PDT [2]. ALA-PDT has already shown encouraging results in the treatment of several skin cancers and diseases [3]. Here the comparison of ALA-PDT effect on leukemia and hepatoma cells was made for exploring the potential of ALA-PDT in different kind cancers.

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MATERIALS AND METHODS

Drugs. ALA and ALA-Hexyl ester (He-ALA), obtained from PhotoCure ASA (Oslo, Norway), were dissolved in the PBS with pH 7.0. The stock solutions of 36 mM were made and kept in 4 ^{0}C before use.

Cells. QGY-7903 human hepatoma cells were obtained from Cell Bank of Chinese Academy of Sciences. The murine WEHI-3B (JCS) myeloid leukemia cells were obtained from John Curtin School of Medical Research, Australia. The cells all were maintained in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS,Gibco), penicillin 100 000 units/l, streptomycin 100 mg/l and 1% glutamine. Cells were incubated at 37°C in a humidified incubator containing 5% CO_2. Cells in the

exponential growth phase were used in each experiment.

Fluorescence measurements. The fluorescence spectra of ALA-incubated cells were measured with a luminescence spectrometer (Carry Eclipse, VARIAN). The excitation wavelength was set at 405 nm (a main absorption peak of PpIX). The fluorescence images of ALA-incubated cells were recorded by OLYMPAS fluorescence microscope equipped with a digital camera (Nikon). The magnification used was 640. The filters for detection of PpIX fluorescence consisted of a 450 nm band pass filter for excitation and a 590 nm long pass filter for obtaining the fluorescence image.

Photodynamic treatment and cell survival assay. The cells were added into 96 wells flat-bottomed culture plates with 2×10^4 cells per well. When attached to the substratum the cells in PDT groups were added with ALA or He-ALA in serum-free medium, and incubated for 5 hours. The cells of both PDT and control groups were subsequently irradiated with different light doses. The light source was a halogen lamp with a heat-isolation filter and a 500 nm long pass filter, as described previously [4]. The fluence rate was 7 mW/cm^2. After light exposure the cells had been incubated with fresh medium containing 10% FCS for 2 days before the cell viability was determined by MTT assay. The details of MTT assay were described previously [4], and the optical density at 540 nm and 690 nm was measured using iEMS Analyzer (Bio-Rad).

RESULTS AND DISCUSSION

Fig 1 shows the fluorescence spectrum of ALA-incubated QGY cells. The typical fluorescence characteristic (635 nm emission peak) reflects that PpIX was produced in cells. PpIX was also found in ALA-incubated JCS cells by its fluorescence (not shown in Fig).

Fig 2 shows the fluorescence image of ALA-incubated JCS cells. Since ALA does not emit fluorescence, the red

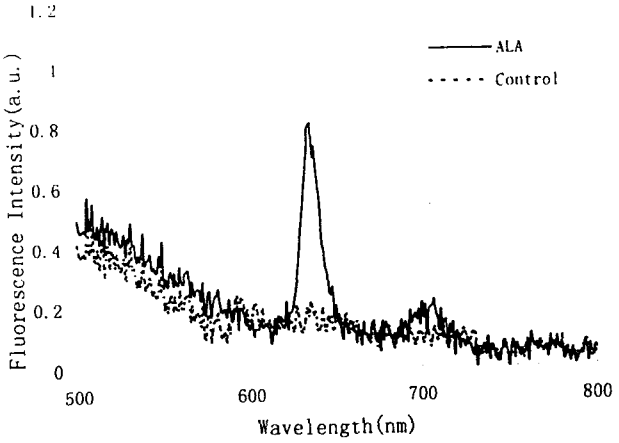

Fig 1. The fluorescence spectrum of ALA-incubated QGY cells. Cells had been incubated with ALA (2mM) for 4 hours and then washed and resuspend in fresh medium with cell density of 10^6 cells/mL. Excitation: 405 nm.

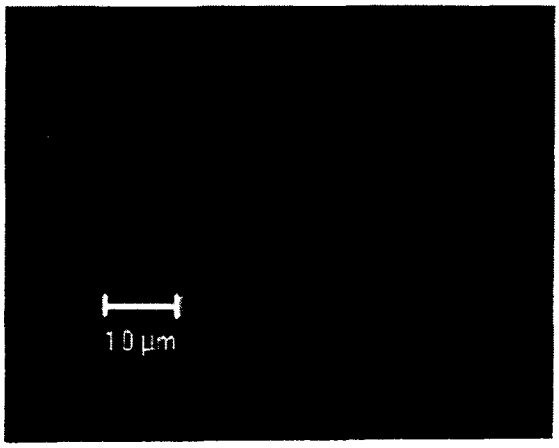

Fig 2. Fluorescence image of ALA-incubated JCS cells. Cells were seeded on the glass slice. Forth-eight hours after seeding, the cells were incubated with ALA (1mM) for 4 hours. Being washed with fresh medium, the slice was picked up for fluorescence imaging measurement.

Table 1. Photo-inactivation of cells by ALA and He-ALA

	QGY		JCS
	ALA (2mM)	He-ALA (0.2 mM)	ALA (1mM)
Light dose	147 kJ/m^2	147 kJ/m^2	45 kJ/m^2
Death Rate	55%	75%	92%

Cells were incubated with ALA or He-ALA for 4 hours, and then irradiated. Cell survival was measured by MTT assay.

Fluorescence in Fig 2 confirms the PpIX formation in cells. It is seen that PpIX is distributed in cytoplasm of JCS cells. PpIX is also found localized in cytoplasm of QGY cells (not shown), reflecting that distribution pattern of PpIX may be similar in different cell lines.

Table 1 shows the photo-inactivation of PDT to QGY and JCS cells. It is obviously that JCS cells are much easy to be destroyed than QGY cells in ALA initiating photosensitization. The results reflect that the efficiency of ALA-PDT is cell line dependant, which may due to the difference of ferrochelatase activity in two cell lines. Thus ALA-PDT may only suit for some kind cancer in which the ferrochelatas activity is very lower and PpIX is easy to be endogenously produced [5], such as JCS leukemia cells. So ALA-PDT showed a big potential to apply in leukemia treatment.

Though QGY cells were not sensitive for ALA-PDT, they were sensitive for He-ALA-PDT. When very low He-ALA incubation concentration (0.2 mM) was used and irradiated with the same dose, the death rate can reached 75%, thus the PDT efficiency was improved. When ALA was esterified, the affinity to the plasma membrane of cells was enhanced. The more He-ALA entered cells, the more PpIX amount formed in cells. It seems that the use of esterified ALA is a good way for improving ALA photosensitization. ALA has different esterified derivatives. Here our results demonstrated that He-ALA, one of ALA esters, is very powerful in QGY photo-inactivation, though the QGY cells die hard in ALA-PDT. He-ALA is a very promising drug, should be explored further in photodynamic therapy.

ACKNOWLEDGEMENTS

We thank NSFC (No 39970186) for supporting this work.

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