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Sensitivity of different cell lines to phototoxic effect of disulfonated chloroaluminium phthalocyanine

H. Kolarova *, R. Lenobel, P. Kolar, M. Strnad

Department of Medical Biophysics, Faculty of Medicine, Laboratory of Growth Regulators, Palacky University, Hnevotínská 3, 775 15 Olomouc, Czech Republic

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Abstract

Photodynamic therapy (PDT) is a treatment for cancer involving three key components: sensitizer, light and tissue oxygen. A sensitizer is a chemical compound that can be excited by light of a specific wavelength. Phthalocyanine $ClAlPcS_2$, belonging among the promising second generation of sensitizers, was evaluated as an inducer of photodamage on NIH3T3 (mouse fibroblasts), B16 (mouse melanoma), MCF7 (human breast adenocarcinoma) and G361 (human melanoma) cell lines. A semiconductor laser was used as a source for evocation of the photodynamic effect. We report the influence of various concentrations of the sensitizer in combination with laser irradiation on the photodamage of cells. Viability of cells was determined by means of molecular probes (Calcein AM and ethidium homodimer) for fluorescence microscopy. The quantitative changes of cell viability in relation to sensitizer concentrations and laser irradiation were proved by fluorometric measurement. We detected phototoxicity evoked by laser irradiated sensitizer in all studied cell lines. In addition, the viability studies showed that G361 melanoma cells and MCF7 breast adenocarcinoma cells were more sensitive than NIH3T3 mouse fibroblasts and B16 mouse melanoma to photodynamic damage induced by $ClAlPcS_2$.

Keywords: Phototoxicity; Cell lines; Laser; Phthalocyanine

1. Introduction

Photodynamic therapy (PDT) is a promising treatment modality that has recently been accepted in clinics as a curative or palliative therapy for cancer and other nonmalignant conditions (Moan and Peng, 2003; Henderson and Dougherty, 1992; Dougherty et al., 1998; Allison et al., 2004). PDT involves the illumination of a photosensitizer with visible light, leading to the production of cytotoxic species such as reactive oxygen species that ultimately induce oxidative biological damage and cell death. Membranous organelles, including mitochondria, plasma membrane, and lysosomes, have been suggested to be the main sites of PDT damage (Kessel et al., 1997; Ben-Hur et al., 1992). Photodynamic treatment also reduced the activity of various enzymes involved in energy metabolism by decreasing the cellular ATP level (Paardekooper et al., 1995).

Apoptosis after PDT has been demonstrated in vitro (Moor, 2000), and in vivo (Zaidi et al., 1994). The initiation of apoptosis after the photodynamic therapy appears to be a function of the sensitizer and the cell line, respectively (Luo and Kessel, 1997). Dyes such as hematoporfyrin derivates for this type of therapy are used. Disadvantage of the hematoporfyrin dyes is that they exist as a mixture of various, mostly unidentified compounds. Moreover, they absorb light at relatively short wavelengths that do

Abbreviations: PDT, photodynamic therapy; $ClAlPcS_2$, disulfonated chloroaluminium phthalocyanine; DMEM, Dulbecco's modified eagle's medium; NIH3T3, mouse fibroblasts; B16, mouse melanoma; MCF7, human breast adenocarcinoma; G361, human melanoma cell lines; IC_{50}, cytotoxic concentration resulting in a 50% reduction in cell viability.

* Corresponding author. Tel.: +420 585632103; fax: +420 5632167.
E-mail address: kol@tunw.upol.cz (H. Kolarova).

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not penetrate deeply into tissue. However, such photosensitizers have important drawbacks including prolonged cutaneous retention and important skin photosensitivity. This invoked the search for new photosensitizers with improved chemical and biological properties. To circumvent these disadvantages, other dyes are tested such as chlorins and phthalocyanines. Phthalocyanines have been extensively examined as photosensitizers for PDT (Martins et al., 2004; Nyman and Hynninen, 2004). These azoporphyrin derivatives have stronger absorbances at longer wavelengths than do porphyrins and tend to have improved photophysical and photochemical properties. Phthalocyanines belonging to the new generation of substances for PDT can be chelated with a variety of metals, chiefly aluminium and zinc, while these diamagnetic metals enhance their phototoxicity (Liu et al., 2004). A ring substitution in phthalocyanines with sulphonated groups will render them water soluble and affect their cellular uptake. Less sulphonated compounds, which are more lipophilic, show the best membrane-penetrating properties and highest biological activity. The kinetics are much more rapid than those of HPD, with high tumour to tissue ratios (8:1) reached after 1–3 h. The phthalocyanines are eliminated much more rapidly, almost without fluorescence seen in the tumour after 24 h (Ometo et al., 1996; Liu et al., 2004; Pandey, 2000; Lacey and Phillips, 2001). In our present study we report the influence of the phthalocyanine concentrations in combination with laser irradiation on the photodamage of different cell lines. The fluorometric measurement with Fluoroscan Ascent was used for definition of cytotoxicity and phototoxicity in vitro.

2. Materials and methods

Sensitizers: Chloroaluminium phthalocyanine $ClAlPcS_2$ was prepared by Jan Rakušan at the Research Institute for Organic Syntheses in Rybitví, Czech Republic. ClAlPc was prepared by the reaction of *o*-phthalodinitrile with aluminium chloride at 200 °C. The product of the reaction was purified by diluted sulfuric acid, washed and dried. The dry ClAlPc was subsequently sulfonated by fuming sulfuric acid. The reaction was drowned into the ice–water mixture. The precipitated disulfonated chloroaluminium phthalocyanine was isolated by filtration. In order to get good solubility, the product was transformed into ammonia salt and in this form used.

Source of light: Semiconductor laser Med 140 (Lasotronic, Ltd. $\lambda = 675$ nm, 21 mW) was used as a source for invocation of the photodynamic effect.

Cell lines (ATTC, USA): NIH3T3 (mouse fibroblasts), B16 (mouse melanoma), MCF7 (human breast adenocarcinoma) and G361 (human melanoma) cell lines were used as a testing system.

Cytotoxicity and phototoxicity measurement: Twice washed tripsinized cells were divided in amount 10^4 to each well of microplate and filled in DMEM with 10% FCS in a total volume of 0.1 mg/ml. After 24 h of cultivation at 37 °C in 5% CO_2 the photosensitiser was added. The final concentration of phthalocyanine ranged from 0.1 to 200 μg/ml in a total volume 0.15 ml. Cells in mixture with photosensitizers were cultivated for 24 h. Cytotoxicity of $ClAlPcS_2$ without irradiation for all cell lines was tested. The values of IC_{50} were obtained. Only the samples in the range from 1 to 10 μg/ml were subsequently irradiated by a laser of wavelength 675 nm at a dose 30 J/cm^2. The quantum of irradiance was measured by Radiometer RK 2500. The controls contained cells in a cultivation medium only. Morphological changes of the cells during cultivation have been observed in an Olympus IX 70 inversion fluorescent microscope. The vitality of cells was evaluated 24 h after irradiation. For identification of live and dead cells, the fluorescent probes ethidium homodimer and calcein were used. The previous results were also proved by flourometric measurement with Fluoroscan Ascent.

3. Results

Cells were irradiated by a laser without addition of photosensitizers. Laser irradiation of wavelength 675 nm at doses in the range from 1 to 150 J/cm^2 was used. Doses of irradiation were selected on the basis preliminary test which confirmed that viability of cells was not decreased under 90% after irradiation doses up to 150 J/cm^2. Cytotoxicity of $ClAlPcS_2$ ranged from 0.1 to 200 μg/ml without irradiation for all cell lines was tested. The viability test proved that the viability was not significantly influenced in the ranges of concentration tested. Viability of all tested cells was not decreased under 90%. We evaluated the influence of $ClAlPcS_2$ concentrations from 1 to 10 μg/ml in combination with laser light irradiation dose at 30 J/cm^2 to viability. (Fig. 1). Phototoxicity measurement showed that all tested cell lines are sensitive to photodynamic damage. In Table 1 the IC_{50} values for all of the tested sensitizers are summarized. Significant influence of the levels of concentration at irradiation dose 30 J/cm^2 was proved ($p < 0.05$), $n = 9$ for each concentration. The values of IC_{50} (50% inhibitory concentration) were estimated by graphical subtraction from dose–response curve using software Microcal Origin (OriginLab® Corporation, Northampton, USA). Dose–response curves were obtained

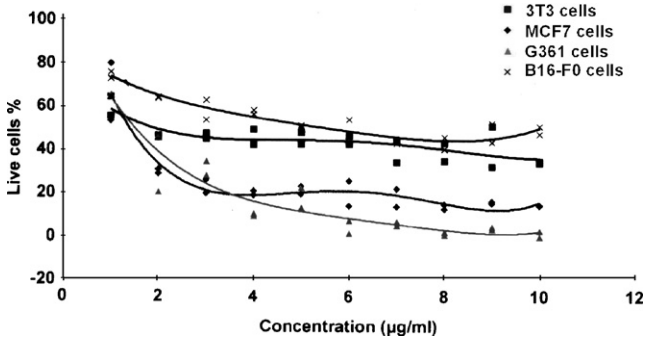

Fig. 1. Dependence of viability on $ClAlPcS_2$ concentrations from 1 to 10 μg/ml in combination with laser light irradiation dose at 30 J/cm^2.

Table 1
Values of IC_{50} [μg/ml]

Cells lines	3T3	MCF7	G361	B16-F0
IC_{50} [μg/ml] $ClAlPcS_2$ without irradiation	>200	>200	>200	>200
IC_{50} [μg/ml] $ClAlPcS_2$ with irradiation dose = 30 J/cm^2	5.6	1.4	1.6	>10
IC_{50} [J/cm^2] without sensitizer	>150	>150	>150	>150

as a dependence of relative ratio of live cells versus a concentration of the tested compound after 60 min incubation of the cells with Calcein AM in a CO_2-incubator at 37 °C and 5% CO_2 (Table 1).

4. Discussion

Efficiency of PDT is affected by various factors including photophysical properties of the sensitizer, wavelength of the activation light, depth of the light penetration in the biological tissue, tissue response on singlet oxygen, etc. Requirements for the choice of the source for invocation of the photodynamic effect are derived from the study of the spectral properties. In the ideal case the wavelength should correspond to one of the absorption maxima of the photosensitizer. The most suitable source of radiation used in PDT is laser. Semiconductor laser with power 50 mW and wavelength 675 nm for invocation of photodynamic effect was chosen, because its wavelength corresponds with the absorption maximum in long-wave region of the visible part of electromagnetic spectra. Penetration of the radiation into tissue is a function of the wavelength. The major sites of PDT damage are membranous organelles, such as mitochondria, lysosomes and plasma membrane. We report the influence of $ClAlPcS_2$ concentrations from 1 to 10 μg/ml in combination with light irradiation doses at 30 J/cm^2 on the photodamage of different cell lines. Viability of tested cells decreases quickly with the increase of phthalocyanine concentration approximately to 4 μg/ml at laser dose of 30 J/cm^2. The higher concentration caused already slight decrease in the viability for all tested cells lines. There are no significant differences between these values of viability. We detected phototoxicity evoked by laser irradiated sensitizer in all studied cell lines. In addition, the viability studies showed that G361 melanoma cells and MCF7 breast adenocarcinoma cells were more sensitive than NIH3T3 mouse fibroblasts and B16 mouse melanoma to photodynamic damage induced by $ClAlPcS_2$.

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