Levels of lipid peroxidation in A549 cells after PDT *in vitro*

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Abstract

Photodynamic therapy (PDT) is an increasingly used treatment for various types of cancer. The principle of PDT involves the administration of a photosensitizer, followed by a distribution interval, and subsequent illumination of tumour area with light of an appropriate wavelength to excite the sensitizer to its triplet state. The aim of the study was to determine the level of lipid peroxidation and the level of thiol groups (-SH) in A549 cells after PDT. The final product of fatty acid peroxidation - malondialdehyde - was quantified

Spectrophotometrically, based on a set of MDA standards of known concentration. Protein damage was based on Ellman's method. The level of lipid peroxidation was significantly higher for cells after PDT, comparing to control cells. We observed much lower concentrations of -SH groups for cells after PDT treatment, in comparison with respective values in control cells. In conclusion, PDT with Ph II induces lipid peroxidation with accompanying protein damage in A549 cells, what can lead to distinct epidemiological, pathological and clinical features.

Key words: photodynamic therapy, photofrin II, lipid peroxidation, thiol groups (-SH).

Introduction

Since its introduction as a promising new method for cancer treatment about two decades ago, photodynamic therapy has become progressively well established as a mode of treatment of malignant as well as non-malignant diseases, characterised by the occurrence of unwanted or harmful cells. Photodynamic therapy (PDT) is an increasingly used treatment for various types of cancer [1, 2]. The principle of PDT involves an administration of a photosensitizer, followed by a distribution interval and subsequent illumination of tumour area with light of an appropriate wavelength to excite the sensitizer to its triplet state. There are several drugs that can be used as photosensitizing agents. The most common of these include ALA, Foscan and Photofrin. Photofrin II, derivative of haematoporphyrin was used in this study. This photosensitizer is taken up into intracellular membranes, mitochondrial membranes, as well as the endoplasmic reticulum and the Golgi system [1, 3].

Photoactivation of Photofrin II is known to generate singlet oxygen and superoxide anion radicals. The ensuing photochemical reaction is dependent on the presence of oxygen, it generates reactive oxygen species (ROS) and induces oxidative stress in the cells, what means tumour destruction [4]. ROS react with thiol groups of proteins and cause damage of cellular membranes. Oxidative stress causes damage to cellular macromolecules, such as nucleic acids, proteins and lipids. Among these targets, peroxidation of lipids is particularly more damaging because the formation of lipid peroxidation products leads to a facile propagation of free radicals. Among the products of lipid peroxidation is malondialdehyde (MDA) - an endogenous genotoxic compound [5, 6].

In this study, we examined the levels of lipid peroxidation and the level of thiol groups (-SH) in A549 cells after PDT.

Material and methods

Cells: human lung carcinoma cell line (A549). This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male.

Cell culture: the cell line was grown in MEM medium with addition of 10% foetal bovine serum. For the experiments, the cells

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were removed by trypsinizing, and washed with PBS. The cells were maintained in a humidified atmosphere at 37°C and 5% CO_2.

Photodynamic treatment: the cells were treated with 15 and 30 µg/ml Photofrin II in complete media 4 h in the dark. Then they were irradiated by the light dose of 6 J/cm^2 during 5 and 10 minutes, using a lamp (OPTEL) with polarized light and red filter (632,8 nm). After irradiation, the cells were incubated in a humidified atmosphere at 37°C and 5% CO_2 for 0, 3 and 6 h. Then the cells were fixed with formaldehyde for 10 min.

Lipid peroxidation: malondialdehyde (MDA), the final product of fatty acid peroxidation, reacts with TBA to form a coloured complex. The level of TBARS was measured on the basis of absorbance at the wavelength of 535 nm. The concentration of malondialdehyde was quantified spectrophotometrically, based on a set of MDA standards of known concentration [7].

Protein damage: protein damage was based on Ellman's method. This method uses reaction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB acid) with thiol groups (-SH) of proteins [7].

Results

The results of MDA concentration are presented in Fig. 1. The level of lipid peroxidation was significantly higher for cells after PDT, comparing to that in the control cells. After a 5-minute irradiation and 15µg/ml of Ph II, we obtained 0,49 µM/l MDA concentration, however, during the same time irradiation but with 30µg/ml of Ph II we got 0,35 µM/l MDA concentration. During a different time period of irradiation (10min) for 15µg/ml and 30µg/ml of Ph II the same MDA concentration (0,5 µM/l) was obtained.

The results of the thiol group (-SH) levels are shown in Fig. 2. We observed a much lower concentration of -SH groups for cells after PDT treatment, in comparison with respective values in the control cells. We obtained over 50nmol/l during 10min irradiation and 30µg/ml of Ph II.

Discussion

The primary objective of this study was to determine oxidative stress in A549 cells that induces PDT. The crucial factors, which determine the type of PDT-mediated cell death, include light dose, the sensitivity of cells and the subcellular distribution of the photosensitizer [4, 5, 6]. In our experimental setting, we studied the levels of the malonyldialdehyde - product of lipid peroxide oxidation in A549 cells, treated by photodynamic therapy, depending on the time and photosensitizer concentration. A high level of lipid peroxidation was found and that was an indication of an increasing, high activity of reactive oxygen species in the environment. Other results verify that lipid peroxidation is one of the photodynamic effects, exerted by hypericin in NPC cells. [8]. Peroxidation of membrane lipids is known to disrupt the membrane structure and induce loss of function, which could lead to cell death [9, 10]. Our investigation confirms earlier studies with PDT on different cell line, showing that, after PDT, the lipid peroxidation level was increased [3, 10]. It has been observed that it depends on different time of irradiation and different dose of photosensitizer. In our studies, we examined different times of irradiation and different concentrations of photosensitizer. We suggest that the best time for irradiation was 5 min for 15µg Ph II for A549 cell line. The higher concentration of Photofrin II requires longer expositions to light. Oxidative stress damage, that affects PDT, is also dependent on cell line and the incubation time with photosensitizing agent before and after exposure to light [4]. Our data represent the first investigation of A549 cellular damage after photodynamic treatment.

Figure 1. The levels of MDA in A549 cells, treated by PDT, comparing to control values.

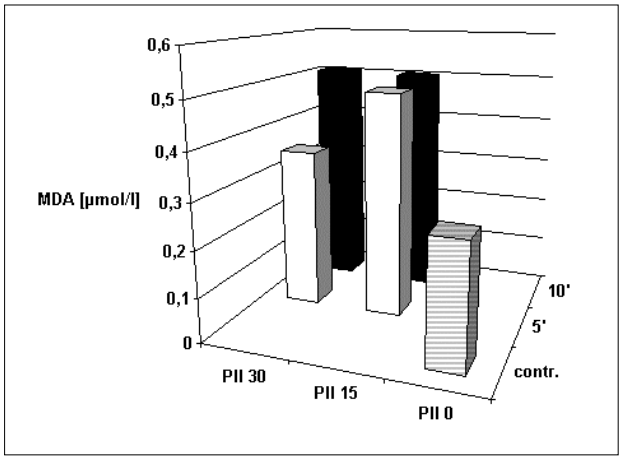
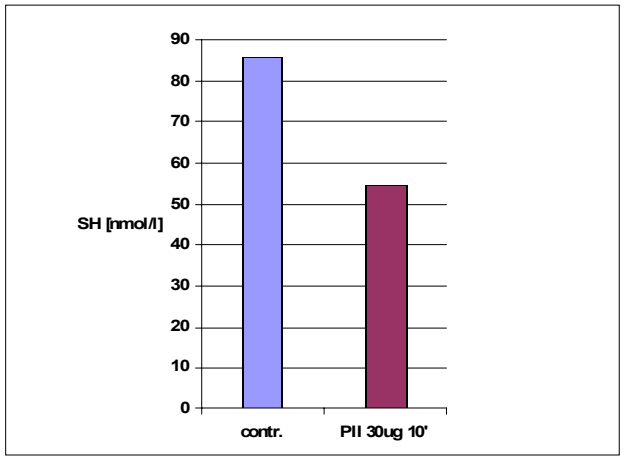


Figure 2. The levels of thiol groups in A549 cells, treated by PDT, in comparison to control values.



For confirmation of our results, also the oxidation of -SH groups was also determined. Our studies revealed that PDT treatment led to membrane damage through protein destruction. Examining single time irradiation and one concentration of Ph II, a distinct decrease of thiol groups concentration was observed [1, 10].

Conclusion

In conclusion, PDT with Ph II induces lipid peroxidation with accompanying protein damage in A549 cells, what can lead to distinct epidemiological, pathological and clinical features. Photofrin II-PDT appears to be an attractive alternative treatment modality for A549 cell line [12].

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