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Photodynamic therapy with TMPyP – Porphyrine induces mitotic catastrophe and microtubule disorganization in HeLa and G361 cells, a comprehensive view of the action of the photosensitizer

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ABSTRACT

Photodynamic therapy (PDT) is a useful tool against cancer and various other diseases. PDT is capable to induce different cell death mechanisms, due to the PDT evoked reactive oxygen species (ROS) production and is dose dependent. It is known that cytoskeleton is responsible for numerous cell functions, including cell division, maintenance of cell shape, their adhesion ability and movement. PDT initiated redistribution and subsequent disintegration of cytoskeletal components that precedes cell death. Here was present our results in HeLa and G361 cells subjected to sublethal PDT treatments using α,β,χ,δ porphyrin-Tetrakis (1-methylpyridinium-4-yl) p-Toluenesulfonate porphyrin (TMPyP). The photosensitizer (PS) induced transient increasing of mitotic index (MI) observable early after PDT, cell cycle arrest, microtubule (MTs) disorganization of interphase cells, aberrant mitosis and formation of rounded cells with partial loss of adherence. Some cells were partly resistant to PDT induced MTs disorganization. The differences between both cell lines to PDT response were described. This is the first evidence of TMPyP - PDT induced microtubule disorganization and the cell death mechanisms known as mitotic catastrophe and the first detail analysis of microtubule aberrations of mitotic and interphase cells in HeLa and G361 cell lines. New modification of techniques of protein immunolabeling was developed.

1. Introduction

The suppression cell division and induction of cell death allow the gradual elimination of tumor cells by autolysis or activation of the immune system and by causing the inflammation process. PDT is one of prospective and less invasive methods used to suppress tumors in clinical practice, where initiation of cell death is based on the application of chemicals called photosensitizers (PS), principally non-toxic photoactive compounds, selectively accumulated in tumor cells and activated by targeted irradiation appropriate wavelength of visible light, which can be directly focused on the treated tissue. Cell death is preceded by damage to the stability of the internal environment of the cells, including the destabilization of chromatin, disruption of cell division, a start of the pathways activation of specific enzymes, kinases and protein degradation initiated by using PDT produced ROS [1–4]. PDT was originally developed as a therapy for tumor suppressions [5,6], but now has a wide application in combating with other non-cancer diseases and with bacterial or fungal infections or overcoming drug resistance [3,7–10]. Many studies are focusing on different types of PS [11,12] and searching for the suitable PS with optimal activation wavelengths of visible light. The absorption maximum of PS is closer to the infrared region, the deeper into the tissue the light is able to penetrate and activate cell death via PDT [3,11]. PDT targets the numerous cellular organelles and components including plasma membrane, mitochondria, Golgi apparatus, lysosomes, [13] and cytoskeleton [14]. More detailed information about the changes of cytoskeleton, cell cycle progression, depending on the type of therapy has been very limited. After PDT treatment, the redistribution and subsequent disintegration of MTs and F-actin arrangement or actin stress fiber formation were recorded as a harbinger of apoptosis induction and cell death initiation. MTs and microfilaments (MFs) are filamentous structures, components of cytoskeleton, which are responsible for cell division, maintenance of cell shape, membrane surface morphology, cell adhesion and movements. It is known that PDT induced alterations in formation of mitotic spindles, aberrant mitosis and/or cytokinesis connected with chromatin instability are followed by cell death. All of these changes occur in according to type of the cell line, used treatment conditions and PSs [15–21]. The PDT induced changes of cytoskeleton were reported mostly for actin MFs [15,22–30], less for intermediate filaments [31–33] and MTs [15,27,32,34,35].

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Alterations in mitotic spindle and in distribution of chromosomes evocate defective mitotic behavior that lead to cell cycle arrest and to a specific cell death process named mitotic catastrophe [36]. The cell cycle arrest is often followed by aneuploidization [16,37,38], which is linked with genomic instability, development of drug resistance, tumor progression and senescence [39–41] and indicate several alterations in check point regulation [42]. The PDT induced cell death could be initiated by different mechanisms, well known is induction of apoptosis or necrosis, but mitotic catastrophe as one of cell death procession is less understood and was described after PDT induced cell death of HeLa cells with using different phthalocyanine derivates [16,43]. In both cases firstly PDT induced cell cycle arrest at metaphase stage that lead to multiple spindle poles formation and finally to cell death [16,43].

Previously we analyzed the effect of PDT of one of the very effective PS TMPyP on several cell lines including HeLa and G361 [9,44]. TMPyP PS with a maximum absorption at 422 nm, belongs to the group of tetrapyrroles, porphyrines and analogues, which are highly effective in medicine and most widely studied compounds in PDT [45–48]. For TMPyP the therapeutic doses, the cell viability and ROS production in dependence to PS concentration and light doses were determined as well as changes in some of early response genes expression and its PDT induced antimicrobial activity [9,44].

This study concentrated to analyses of the complex effect of TMPyP PS on MTs of interphase and mitotic cells, cell cycle progression of HeLa and G361 cells, the morphological alterations on the mitotic spindle and we also evaluated its implication in cell death.

2. Materials and Methods

2.1. Preparation of Cell Culture

The HeLa (human cervix adenocarcinoma) and G361 (human melanoma) cells were grown on coverslips 22 × 22 mm in sterile 6-well microplates or suitable small Petridishes, (100,000 cells/well or Petridishe) - (TPP Techno Plastic Products AG, Switzerland) in the presence of cultivation medium DMEM (Dulbecco's Modified Eagle Medium, with 10% fetal bovine serum and supplied by penicillin, streptomycin and glutamine), (Sigma-Aldrich, USA). Cell cultures were incubated at 37 °C and in 5% CO_2 in CO_2 incubator (Sheldon Manufacturing, Inc., USA).

2.2. Photodynamic Treatment - Application of PDT

Asynchronous cells at above mentioned concentration were incubated 24 h in DMEM incubation medium, then the PS α,β,χ,δ porphyrin-Tetrakis (1-methylpyridinium-4-yl) p-Toluenesulfonate porphyrin (TMPyP) - (Sigma-Aldrich, USA), diluted in DMEM was added at the final concentration 2,5 μM. TMPyP concentration and irradiation dose for PDT treatment of HeLa and for G361 cells were chosen as a sublethal doses during studied intervals on the base of previous published results of MTT tests (cell viability assays) for the same cell lines [9,44] and our previous MTT tests for G361 cells that showed surviving of the cells with using of different concentrations of PS (Fig. 1A) and different irradiation doses [data not shown]. Cells with applied PS were incubated for the next 24 h in common cultivation conditions in darkness as was described above. Then the PS was rinsed with PBS buffer (10 mM Na_2HPO_4, 1,8 mM KH_2PO_4, 137 mM NaCl, 2,7 mM KCl, pH 7,4) and replaced by fresh cultivation medium - phenol red free DMEM (Sigma-Aldrich, USA). The cells were continuously irradiated by specialized light emitting diodes (LEDs), with excitation wavelength 414 nm; full width at half maximum [FWHM]: 15 nm; intensity: (10 mW/cm^2) at the total light dose 1 J/cm^2, total time of irradiation was 20 s. Irradiance was measured by the radiometer system IL 1705 (International Light Technologies, USA). When irradiation was finished, post PDT incubation time was measured. For HeLa cell line the PDT effects were studied at post PDT time (time after irradiation) 0 min, 5 min, 10 min, 20 min, 60 min and 120 min, for G361 cell line were used the same post PDT intervals except 5 min. Moreover G361 cell line was analyzed also at interval 24 h post PDT. As controls were used cells cultivated without any treatment, cells at the same light conditions and post irradiation time but without PS, and cells with the same dose of PS incubated only in dark.

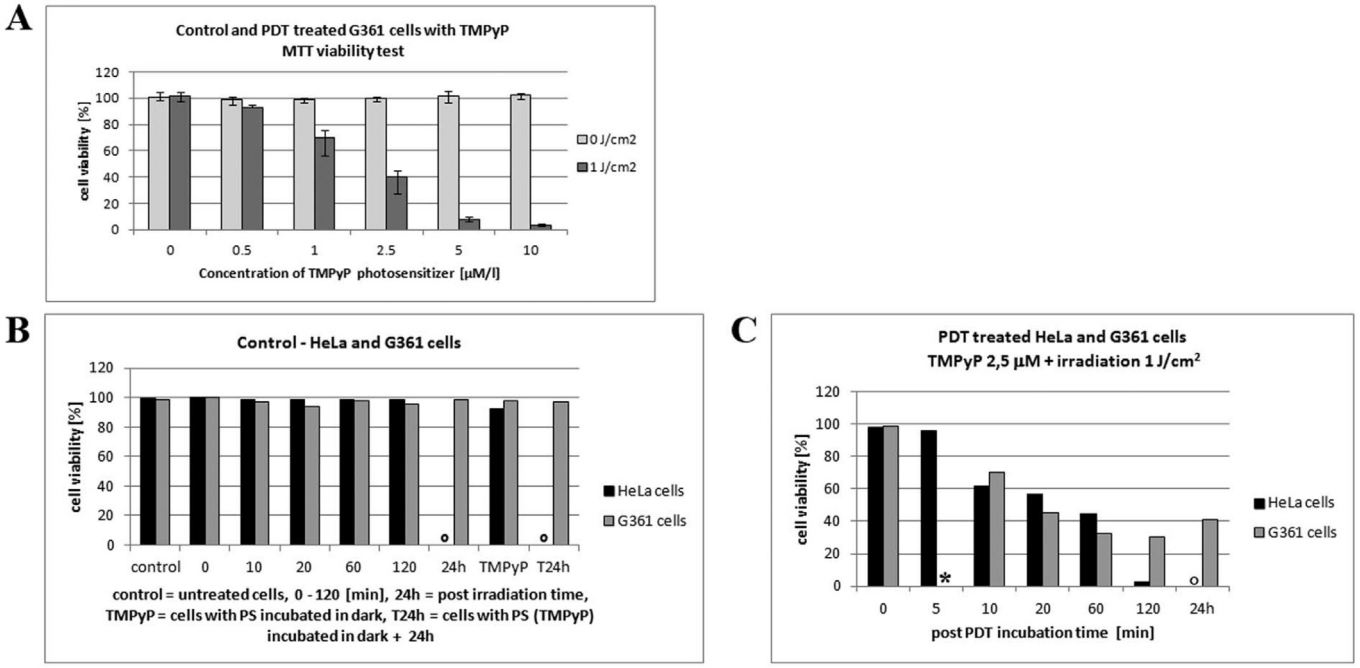

Fig. 1. Cell viability of HeLa and G361 cell lines; A - MTT test - G361 PDT treated cells as compare to control for different PS concentrations. B,C - Cell viability of HeLa and G361 cell lines, results from immunolabeled slides analysis; B - control cells (control - untreated cells, 0, 10, 20, 60, 120 [min], 24 h - cells without PS at certain time after irradiation 1 J/cm^2, TMPyP – cells incubated with PS (2,5 μM TMPyP) in dark, T24 h - cells incubated at first with PS (2,5 μM TMPyP) 24 h and then incubated for a further 24 h, after washout of the PS from cell culture, in dark under common cultivation conditions), C – TMPyP - PDT treated cells, (0, 10, 20, 60, 120 [min], 24 h - post PDT time), * - the cell viability 5 min after PDT was not analyzed for G361 cells, ° - the cell viability after 24 h was not analyzed for HeLa cells.