PHOTODYNAMIC THERAPY OF MEDULLOBLASTOMA *IN VITRO* EXPLORED USING FLUORESCENCE MICROSCOPY

Kimberly F. Ingersoll[1], Usiakimi Igbaseimokumo, MD[2], Scott N. Litofsky, MD[2] and G. Esteban Fernandez, PhD[3]

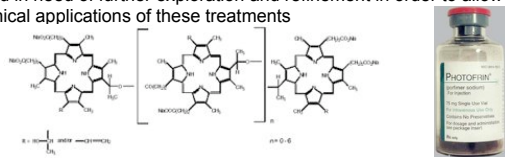
[1] University of Missouri Medical School, [2]Division of Neurological Surgery, [3]Molecular Cytology Core

Abstract

There is very little application of photodynamic therapy to pediatric brain tumors, thus it is important to explore the localization of the commonly used photosensitizer Photofrin in a novel cell line *in vitro*. To date there are no studies quantifying the optimal concentrations and uptake of Photofrin by medulloblastoma cell lines. Various concentrations of Photofrin and incubation times will be explored to determine the optimal concentrations and incubation period for Photofrin *in vitro* and where the Photofrin is sequestered within the cells. Cellular localization will be assessed using fluorescence microscopy and the relative fluorescence intensity of the cells will be quantified.

Background/Introduction

- PDT: FDA-approved to treat some skin and esophageal cancers
- Photosensitizer administered to a patient, tumor irradiated with light of a certain wavelength
- Tumor cells retain the photosensitizer longer than normal tissue, thus PDT selectively kills cancer cells
- Mechanism of cell death: superoxide mediated, leading to apoptosis
- The application of PDT to a variety of brain cancers is a promising field in need of further exploration and refinement in order to allow clinical applications of these treatments



- Photofrin: photosensitizer that has been approved for clinical PDT use in certain cancers
- Photofrin: mixture of porphyrin oligomers, activated at 408 nm, fluoresces at 630 nm
- Depending on the hydrophobicity and charge of the photosensitizer, the localization of a photosensitizer may vary
- Photofrin diffusely localizes to the cytoplasm in other cell lines
- Diffuse cytoplasmic localization correlates with the most effective PDT *in vitro*

Materials and Methods

- Daoy cells (ATCC) cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% Fetal Bovine Serum (FBS)
- $1x10^4$ to $1x10^5$ cells plated to each well of a chambered coverglass
- PF-doped medium added to the cells (0 – 20 μg/mL) for various incubation times (0 – 48 hours)
- Zeiss LSM 5 live line-scanning confocal microscope (λ_{ex} = 405 nm, λ_{em} = 630 nm, power = 150 μW, 333 ms duration, long-pass filter)
- MetaMorph software to quantify fluorescence
- Controls: cell control (no PF), medium control (no cells or PF), PF control (no cells)

Results

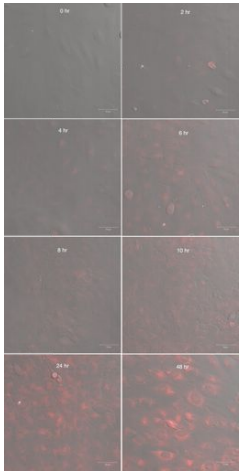


Figure 1: Fluorescence microscopy images of Photofrin localization in Daoy cell line at various incubation times in 20 μg/mL Photofrin-doped growing medium (20x magnfication). Scale=50 μm.

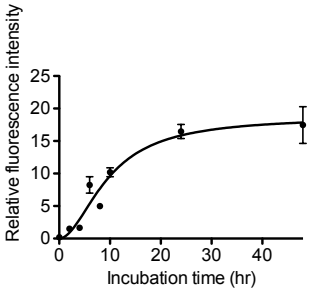


Figure 2: Incubation time dependence of the relative fluorescence intensity of the Daoy cell line treated with 20 μg/mL Photofrin. The optimal incubation time for the Daoy cell line in Photofrin is 24 hours. Error bars represent the standard deviation at each point.

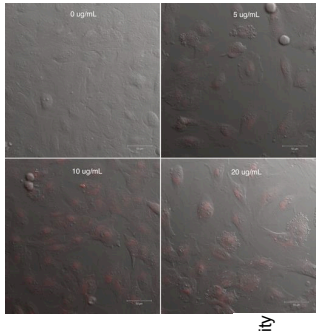
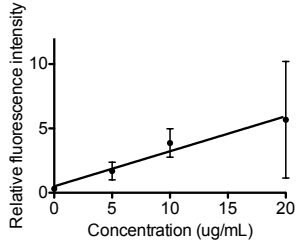


Figure 3: Fluorescence microscopy images of Photofrin localization in the Daoy cell line at various concentrations of Photofrin-doped growing medium incubated for 24 hours (20x magnification). Scale = 50 μm.

Figure 4: Concentration dependence of the fluorescence intensity of the Daoy cell line incubated with Photofrin for 24 hours. The scatter-plot shows that fluorescence intensity is directly proportional to concentration (y = 0.2723x+ 0.5050). Error bars represent the standard deviation at each point.



Conclusions

- Medulloblastoma cells are able to take up Photofrin in culture (no lymph support cells required), thus the cells are intrinsically capable of absorbing Photofrin
- Photofrin exhibits perinuclear localization
- Future work with other cell lines (vascular endothelial cells, astrocytes) and *in vivo* studies with nude mice
- PDT shows promise to detect and treat medulloblastoma tumors

Acknowledgements

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