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Sarah Rogers
Ouachita Baptist University

Joseph E. Bradshaw
Ouachita Baptist University

Timothy E. Hayes
Ouachita Baptist University

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Porphyrin Derivatives and Photodynamic Therapy Effects on Triple Negative Breast Cancer

*Sarah Rogers[1], Joseph E. Bradshaw, PhD[2] and Timothy E. Hayes, PhD[2]
Departments of Biology[1] and Chemistry[2], Ouachita Baptist University
Arkadelphia, AR 71998

Abstract

There are limited effective options for treatment of triple negative breast cancer (TNBC) due to its lack of the three receptors typically used to target breast cancer. The use of photodynamic therapy (PDT) to kill cells that take up light-absorbing compounds (PDT agents) may be an effective option to treat TNBC. We tested the efficacy of modified porphyrins as PDT agents against cells from TNBC. We compared these to Foscan, which is similar in structure to porphyrins and has been approved for use in Europe. Our 1st goal was to measure which porphyrins were taken up best by TNBC cells. Measuring the uptake of some of our compounds had been problematic due to their hydrophobic nature. We optimized the uptake protocol and showed that TNBC cells take up the compounds to different extents. One of the primary side effects of PDT is skin toxicity for up to 4-6 weeks after treatment due to exposure to sunlight. Our 2nd goal was to compare the toxicity in the light and in the dark of PipOH, H_2TPPC, and Foscan. In previous experiments, Foscan showed dark toxicity at low concentrations, but in these experiments there was variability in our results with Foscan so no clear comparison could be drawn. Our 3rd goal was to find combinations of PDT agent and concentration that are effective on TNBC cells at high light energy but minimize killing cells with ambient light. We measured the effect on cell killing by varying both the light dose and the concentration of 3 compounds to find concentrations that are effective at high doses of light but minimize toxicity at moderate doses. All 3 compounds show promise, but the dose must be carefully selected.

Introduction

There are limited effective options for treatment of triple negative breast cancer (TNBC) due to its lack of three receptors typically used to target breast cancer- the estrogen receptor, the progesterone receptor and the human epidermal growth factor receptor-2. The lack of these targets makes treatment difficult since patients with TNBC are not candidates for hormone therapy or trastuzumab-based regimens, which works on HER2. Current treatments for TNBC result in poor overall survival no matter the stage with a rapid increase in the risk of recurrence at 1-3 years. Better treatment options are essential to ensure better treatment and survival rates. People of younger age, African American race, younger age at menarche, strong family link to breast cancer, and those with breast cancer 1 early onset (BRCA1) mutation are at an increased risk for developing TNBC.

Photodynamic therapy (PDT) is a form of therapy used to kill tumors. A photosensitizer (PS) is applied to the area of treatment. Exposing the PS to light produces a reaction with oxygen that kills both tumors and vasculature. Foscan is a PS that has been approved for use in Europe and is highly effective for some types of tumors. Use of this drug necessitates that patients stay in the dark for 24 hours after treatment because exposure to ambient light may cause burns. One purpose of the current study is to find a combination of porphyrin concentration and light dose that minimizes the unwanted side effects of skin sensitivity.

One of the properties that determine how active a PDT agent will be is how well it is taken up by cells. Transport in the blood and uptake by cells require a balance between the hydrophilic and hydrophobic nature of the porphyrins. We have had difficulty measuring uptake of some porphyrins because they stick to the plastic plates used to grow the cells. To accurately measure uptake, it is important to find methods to reduce background binding.

Goals and Objectives

Goal- To find a better treatment method for triple negative breast cancer.

Objectives:

- Measure how well the porphyrin derivatives are taken up by TNBC cells
- Find a combination of light dose and porphyrin concentration that kills cells at higher doses of light but not at moderate doses.

Materials and Methods

Cell culture- MDA-MB 231 TNBC cells were grown using published protocols. For each experiment, two sets of 96-well plates were needed- one exposed to light and one kept in the dark. Varying concentrations of porphyrins were applied to the wells in growth medium in the dark, and the plates were returned to the 37°C incubator. 8 wells were used for each condition. After 24 hours, medium from both plates was replaced with fresh culture medium. One plate of cells was exposed to white light at (0.5 J/cm²) and both plates were cultured in the dark for 3 days at 37°C. An MTT assay for cell proliferation was performed on the TNBC cells and absorbance was read on a microplate reader at a 570 nm wavelength with a 630 nm correction. Values from replicate wells were used to calculate the average and standard deviation. % viability was calculated by comparison to the average of the untreated wells.

Light dose- 4 plates were grown with cells and treated identically with different porphyrin concentrations. After 24 hours, medium with porphyrin was removed from the cells and replaced with fresh growth medium. 3 of the plates were exposed to varying amounts of light- 0.5, 5, or 15 J/cm²- and returned to the 37°C incubator for 3 days in the dark. An MTT assay was performed on the 96-well plates to measure viability.

Uptake- Two 24-well plates were prepared, one with cells and a parallel plate with no cells, to be treated with porphyrins. Porphyrins were diluted into fresh growth medium supplemented with 0.01% BSA. 0.5 ml of each dilution was added to each of 6 wells-3 wells with and 3 wells without cells. The plates were incubated at 37°C in the dark. After 24 hours, medium was aspirated from the wells and the cells washed 2x with 1ml of PBS. 250 µl of 0.25% Triton X 100 in PBS was added to each well and then incubated at 37°C in the dark. After 1 hour, the plates were shaken for 15 minutes at room temperature. 200 µl was removed from each well and transferred to a clean, labeled tube. The tubes were centrifuged for 3 minutes at 16,000 x g. A fluorescence assay was performed on aliquots of the supernatant, and the remainder of the samples was frozen until the protein assay. A standard curve was prepared by diluting a porphyrin standard solution into 0.25% Triton X 100 in PBS. The fluorescence of each dilution was measured by exciting the wells at 415 nm and reading the emission at 650 nm. The standard curve was used to find the amount of porphyrin in each well. Protein assays were performed on the supernatants using the Bradford protocol.

Porphyrins- the porphyrins used in this research were synthesized in the lab of Joe Bradshaw. PipOH, H_2TPPC, and H_2TPPSO were made by adding different side chains with varying hydrophobic and hydrophilic properties to the R positions of the porphyrin ring structure. Foscan has a chlorin ring which is similar to the porphyrin ring except that one of the C=C double bonds in one of the pyrrole rings is reduced to a single bond.

Porphyrin ring structure **R groups:**

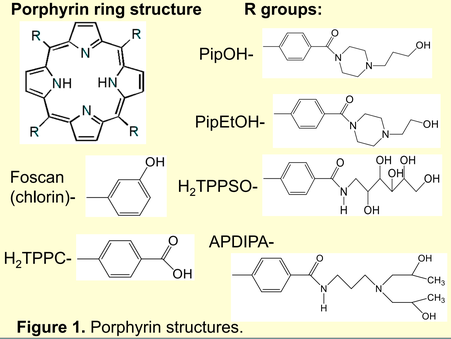

Figure 1. Porphyrin structures.

Results

Uptake: For some porphyrins, binding to cells was a modest fraction of total binding in uptake experiments. Two methods were tried to increase the ratio of the specific binding versus background binding of the porphyrin to the plastic wells. Pre-treating the wells with detergents or proteins before cells were added was not effective (data not shown). The second method we tried was mixing detergents or proteins with growth medium before adding the porphyrin.

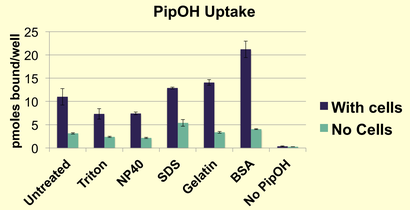

Figure 2. Reagents to reduce background binding. Detergents (Triton X100- 0.004%, NP-40- 0.001%, SDS- 0.01%) and proteins (BSA- 0.01%, Gelatin- 0.01%) were mixed in growth medium before porphyrin was added. Parallel plates, one with MDA-MB 231 TNBC cells and one without cells, were treated with the porphyrin solutions (3 wells per condition) for 24 hours and uptake was measured. Error bars indicate standard deviations.

Results- Some reagents produced a significant increase in binding to the cells compared to the wells. BSA appeared to have the greatest effect. It is possible that the reagents increase specific binding by reducing aggregation of the hydrophobic porphyrin derivatives.

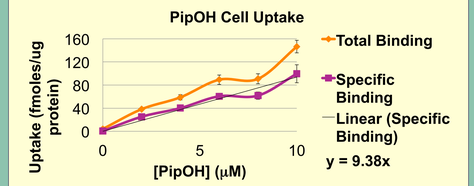

Figure 3. Dependence of PipOH uptake on concentration. 0.01% BSA was mixed into growth medium before porphyrin was added. Medium with porphyrin was added to parallel plates, one with MDA-MB 231 TNBC cells and one without cells (3 wells per condition). After 24 hours of incubation, PipOH uptake was assayed by fluorescence, and protein assays were performed on the dissolved cells. Standard deviations from triplicate wells are shown by error bars.

Results- As the concentration of PipOH increases, the amount of porphyrin taken up by the cells increases in a linear fashion. Most of the binding is to the cells. The slope of the regression line is a measure of the affinity of binding. The affinity of PipOH is similar to but slightly better than that of the previously tested porphyrins (see Table I).

Porphyrin	Specific Binding (fmoles/ug/uM)
PipOH	9.4
PipEtOH	6.8
APDIPA	3
H_2TPPSO	2.5
Foscan	138-459

Table 1. Specific binding of porphyrins to MDA-MB 231 TNBC cells.

MTT assay- In the MTT assay, viable cells oxidize the water-soluble yellow MTT to a purple insoluble formazan. The purple precipitate is dissolved in DMSO and quantitated by absorbance. More purple color indicates more viable cells.

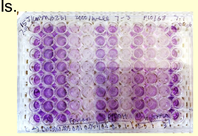

Figure 5- A 96-well plate showing variances in purple color indicating differences in cell viability.

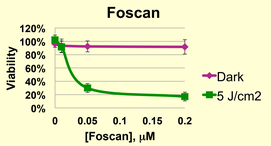

Figure 6- Effects of porphyrin concentration on TNBC cell viability in the dark and with exposure to light.

Results- Foscan shows concentration-dependent killing of TNBC cells if they are exposed to light. In the dark, Foscan shows little toxicity at these doses.

Light Dose- MDA-MB 231 TNBC cells were grown on 4 96-well plates for 3 days before treating with varying concentrations of porphyrin. After incubation for 24 hours, the plates were exposed to varying light doses (0.5, 5, and 15 J/cm²) with 1 plate kept in the dark. After 3 more days, wells were assayed for viability using an MTT assay.

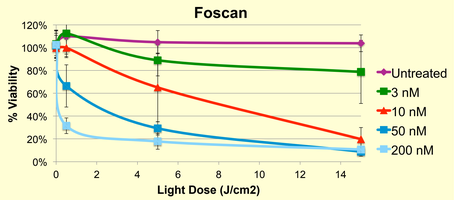

Figure 7. Effect of light dose on viability at varying concentrations of Foscan (average of three experiments).

Result- At 10 nM, Foscan shows high viability up to 5 J/cm² but significant toxicity at 15 J/cm².

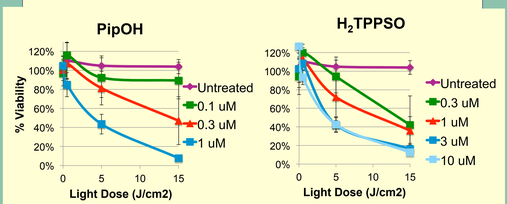

Figure 8. Effect of light dose on viability at varying concentrations of PipOH and H_2TPPSO (average of three experiments).

Results- There are concentrations of both PipOH (0.3 µM) and H_2TPPSO (1 µM) which show fairly high viability at light doses up to 5 J/cm² but increased toxicity at 15 J/cm². However, both show similar toxicity at 5 J/cm² or lower toxicity at 15 J/cm² than does 10 nM Foscan.

Discussion

We were able to overcome low specific binding of PipOH to cells by adding BSA to the growth medium prior to adding the PipOH. This enabled us to measure affinity of PipOH for TNBC cells. Additional experiments are underway to measure the uptake of Foscan and H_2TPPC. We are unsure the exact extent to which Foscan is taken up by the cells, but we do know that it is taken up much better than any other porphyrin we have tested so far. Our results from the light dose experiment seem promising in finding a combination of light dose and porphyrin concentration that minimize unwanted skin toxicity from ambient light. At 10 nM, Foscan shows high viability up to 5 J/cm² , but significant toxicity at 15 J/cm². PipOH and H_2TPPSO show a similar pattern but have less separation between viability at moderate and higher doses of light. Higher doses of light in the clinical range of 50-200 J/cm² need to be tested.

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