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Potophyrin Derivatives and Photodynamic Therapy Effects on Tripe Negative Breast Cancer

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Abstract

There are limited effective options for treatment of triple negative breast cancer (TNBC) due to its lack of receptors for the three agents typically used to target breast cancer: the estrogen receptor, the progesterone receptor, and the human epidermal growth factor receptor-2 (HER2). These targets make 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 what the stage with a rise in the risk of recurrence at 1.3 years. Better treatment options are essential to ensure better treatment and survival rates.

Introduction

Photodynamic therapy (PDT) is a form of therapy used to kill tumors. A photosensitizer (PS) is applied to the tumor site in the dark. In the presence of light, the PS absorbs energy and reacts with oxygen to form reactive oxygen species that kill the cell. It is effective for treating tumors that are not well supplied with blood, and is effective for some types of tumors. Use of this drug necessitates that patients stay in the dark for 24 hours prior to treatment because exposure to ambient light can cause burns. One purpose of the current study is to find a correlation between photodynamic 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 porphyrin. We have had difficulty measuring uptake of some porphyrins in cell culture so 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 allows cells to grow at higher doses of light but not at moderate doses.

Materials and Methods

Cell culture-MDA-231 TNBC cells were grown using published protocols. For each experiment, two sets of 96-well plates were used. In one set, the plates were washed with medium, 100 µl of porphyrin was added to the wells in growth medium in the dark, and the plates were returned to the incubator. In the second set, the plates 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 varying amounts of light using a medium containing porphyrins. Pre-treating the wells with light and light-sensitizing porphyrins before cells were added was not effective (data not shown). The second method was tried by mixing different porphyrins or different concentrations of porphyrin at moderate doses of light but lower toxicity at moderate doses. All 3 compounds show promise, but the dose must be carefully selected.

Results

SARAH ROGERS, J. E. BRADSHAW, AND T. E. HAYES

Porphyrin–the porphyrins used in this research were synthesized in the lab of Joe Bradshaw. PipOH, HTPPC, and H2TPPSO were made by adding different side chains with varying hydrophobic and hydrophilic properties to the porphyrin positions of the porphyrin ring structure. Foscan has a choline ring which is similar to the porphyrin ring except that one of the C=C double bonds in one of the porphyrine rings is reduced to a single bond.

Porphyrin ring structure R groups:

- Foscan
- PipOH
- HTPPC
- H2TPPSO

Foscan (choline)

Results-For some porphyrin, binding to cells was 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 with growth medium before adding porphyrin resulted in variability in our results with Foscan so we decided to use an alternative approach. The second method we tried was mixing different porphyrins or different concentrations of porphyrin at moderate doses of light but lower toxicity at moderate doses. All 3 compounds show promise, but the dose must be carefully selected.

Discussion

We were able to overcome low specific binding of PipOH to cells by adding BSA to the growth medium prior to adding the PS. This enabled us to measure affinity of PipOH for TNBC cells. Additional experiments are underway to measure the uptake of Foscan and HTPPC.

Acknowledgements

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Figure 1. Porphyrin structures.

Figure 2. Figure 3. Dependence of PipOH uptake on concentration. 0.01% BSA was mixed into growth medium before addition. 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 on the dissolved cells. Standard deviations from triplicate wells are shown by error bars. Results-As the concentration of PipOH increases, the affinity of porphyrin taken up by TNBC cells increases in a linear fashion. Most of the binding is to the cells. The slope of the regression lines shows the strength of binding. The affinity of PipOH is similar to but slightly better than that of the previously tested porphyrins (see Table 1).

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

<table>
<thead>
<tr>
<th>Porphyrin (mM)</th>
<th>Binding (%)</th>
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<tbody>
<tr>
<td>PipOH 100%</td>
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<tr>
<td>PipOH 10%</td>
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<tr>
<td>PipOH 5%</td>
<td></td>
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<tr>
<td>PipOH 2%</td>
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<tr>
<td>PipOH 1%</td>
<td></td>
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<tr>
<td>PipOH 0.5%</td>
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<td>PipOH 0.1%</td>
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Figure 4. Effect of light dose on viability at varying concentrations of Foscan (average of three experiments). Results-At 10 mM, Foscan shows high viability up to 5 J/cm² but significant toxicity at 15 J/cm².

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

Figure 6. Results-As the concentration of PipOH increases, the affinity of porphyrin taken up by TNBC cells increases in a linear fashion. Most of the binding is to the cells. The slope of the regression lines shows the strength of binding. The affinity of PipOH is similar to but slightly better than that of the previously tested porphyrins (see Table 1).

Figure 7. Effect of light dose on viability at varying concentrations of Foscan (average of three experiments). Results-At 10 mM, 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 HTPPC (average of three experiments). Results-There are concentrations of both PipOH (0.3 µM) and HTPPC (1 µM) which allow for moderate light doses of up to 5 J/cm² but increased toxicity at 15 J/cm². Foscan and PipOH show similar pattern but have less separation between viability and higher doses of light. Higher doses of PipOH and HTPPC are needed to test the clinical range of 50-200 J/cm².