

MASTER'S THESIS

A mechanistic study on the photodynamic effects of pyropheophorbide-a methyl ester (MPPa) on prostate cancer PC-3M

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**A Mechanistic Study on the Photodynamic Effects of
Pyropheophorbide-*a* Methyl Ester (MPPa) on Prostate Cancer
PC-3M**

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**A thesis submitted in partial fulfillment of the requirements
for the degree of
Master of Philosophy**

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Abstract

Photodynamic therapy is a promising treatment for cancer and other nonmalignant conditions, which involves the administration of a photosensitizing agent followed by exposure of the tissue to visible nonthermal light (400-760 nm). Photosensitizers are often taken up by malignant or dysplastic tissues with some selectivity, and light delivery can be targeted to the appropriate tissue. When the photosensitizer is illuminated with light of the appropriate wavelength, the molecule is excited. This produces a series of energy transfers leading to the liberation of singlet oxygen, a highly reactive and cytotoxic species, resulting in cell death. The combination of drug uptake in malignant tissues and selective light delivery has the potential to provide an effective tumor therapy with efficient cytotoxicity and limited damage to the surrounding normal tissue.

The first drug to be accepted by the FDA was Photofrin (trademark). Photofrin is a clinically used drug based on Hematoporphyrin derivatives (HPD). Although Photofrin is the most commonly used photosensitizer, it has significant side effects. Therefore, major effort has been invested in the development of new sensitizers. The objective of this study is to investigate the *in vitro* and *in vivo* efficacy of MPPa mediated photodynamic treatment of human prostate cancer PC-3M.

1. In the *in vitro* study, we aim to examine the photocytotoxicity of MPPa induced PC-3M cell death. PDT-induced apoptotic cell death has previously been shown in many cell lines. In this study, the mode of MPPa induced apoptotic cell death was also investigated.
2. In the *in vivo* study, we aim to establish an animal tumor model to evaluate the *in vivo* photodynamic efficacy of MPPa. The results would be very useful in the future application of this compound clinically.

In vitro study, the photocytotoxicity of MPPa in PC-3M cells showed a light- and drug-dose dependent manner and a low photodynamic dose (PD₅₀) was required to produce 50% cell killing. In addition, no significant dark cytotoxicity was observed at the dose range of 0.25 to 8μM. Experiments on MPPa-induced apoptosis were performed under the conditions of LC₇₅(2μM+55.6kJ/m²). By determining activities of caspase-3, 8, 9, we found MPPa mediated PDT induced apoptosis mainly via the mitochondrial/Casp-9/Casp-3 pathway. By the method of flow cytometry, we found the percent distribution of cells in G₀/G₁-phases decreased and G₂/M-phases increased obviously after MPPa mediated PDT while the percentage of cells in S-phase decreased slightly. These findings suggest that MPPa restrain the cell cycle progression from the more sensitive G₀/G₁-phases and led the fate of these cells to apoptosis. Cells in G₀/G₁-phases are sensitive to PDT, maybe because cells in S-phase and G₂/M-phases are busy synthesizing DNA and mitosing and uptake less MPPa than cells in G₀/G₁-phases. *In vivo* study, compared with the

control group, the growth of the implanted tumors was significantly inhibited, with reduced weight and volume, and the tumor volume and weight inhibition rate was 78.66% and 72.07% respectively. Slices of PC-3M tumor after PDT under light microscope and transmission electron microscope showed many apoptotic cells with nuclei condensation and more eosinophilic cytoplasm. Few necrotic cells can be seen with the characteristic morphologic changes of cell injury, including cell swelling and rupture (loss of nuclear staining or karyolysis).

From the above experimental results, it is concluded that MPPa has a noticeable effect on PC-3M tumor. MPPa-mediated photodynamic therapy was an efficient therapy and is expected to be suitable for the treatment of human prostate cancer.

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