

## MASTER'S THESIS

### Photodynamic therapy of merocyanine 540 and temoporfin mTHPC on neuroblastoma neuro-2A

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Photodynamic Therapy of Merocyanine 540 and  
Temoporfin mTHPC on Neuroblastoma Neuro-2A

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## Abstract

Photodynamic therapy is referred to as the specific damage of tissue by a special type of chemicals when excited by the visible light. It is one of the effective cancer treatment methods. The chemicals employed are called photosensitizers because they are sensitive and reactive to light. The success of this cancer treatment method is due to the two special properties of the photosensitizers. (i) The non-toxic property of the photosensitizers: It is non-toxic to the living tissue in the absence of light irradiation. (ii) The tissue selectivity: The photosensitizers accumulate at a higher level in malignant tissue than in normal tissue. On photoactivation, the photosensitizers damage the cell either through a free radical dependent (Type I) reaction or a reactive oxygen dependent (Type II) cytotoxicity action.

MC540 is a negatively charged membrane-bound photosensitizer. It was originally discovered as an effective photosensitizer in the photoinhibition of some cancer such as leukemia and lung cancer. MC540 has also been shown to photokill microorganisms such as enveloped virus and herpes simplex virus. The possible application of MC540 in the killing of other tumor cells has also been recently examined. mTHPC is a second generation photosensitizer which has a number of properties to make it an ideal photosensitizer. It has a high lipophilicity, strong absorption at the region of red light, and more importantly, the high selectivity toward tumor cells. In the present study, the cytotoxicity, uptake kinetics and sensitization effects of the MC540 and mTHPC on the Neuroblastoma cells were investigated. Attempts were also made to study the mode of tumor cell killing of mTHPC. To further examine the tissue selectivity of the sensitizer, an *in vitro* system of primary cultured normal rat spinal cord neurons was set up and evaluated.

In the absence of light irradiation, significant cytotoxicity was not observed for the two photosensitizers on the Neuroblastoma Neuro-2A cells. Both MC540 and mTHPC was found to mediate photokilling effect on the Neuro-2A cells. The two photosensitizers killing the Neuro-2A cells in a drug-dose and light-dose dependent manner. In the sensitization kinetic studies, the peak cytotoxicity of MC540 (40  $\mu\text{g/ml}$ , at the light dose 80  $\text{kJ/m}^2$ ) was observed at two hours after the drug treatment. However, maximum cytotoxicity was observed after exposure of Neuro-2A to mTHPC for 16 hours (1.0  $\mu\text{g/ml}$ , at the light dose 80  $\text{kJ/m}^2$ ). Under these experimental conditions, the

maximum photocytotoxicity for the two photosensitizers on Neuro-2A cell were around 80 %. Using the limiting dilution assay, the frequency of proliferative cells after MC540- and mTHPC-PDT were found to be lower than 1 in 1000. This indicates that over 99.9% of Neuro-2A cells are irreversibly damaged by MC540 and mTHPC.

The mode of cytotoxicity of mTHPC-PDT on Neuro-2A was examined. Significant apoptotic cell death was not observed after 24 hours after PDT. Results from immunostaining revealed that the expression of, apoptotic suppressing protein Bcl-2 was found to be upregulated in mTHPC-PDT treated Neuro-2A cells. Genotoxic effect of the mTHPC was also studied using the comet assay. Under the experimental PDT conditions (LD50 and LD80). Significant genotoxicity was not observed at 4 hours and 24 hours after light treatment. Furthermore, the mTHPC-mediated cytotoxicity activity on Neuro-2A cells was found to be reduced by the reduced by the singlet oxygen scavenger (DPBF), indicating that singlet oxygen is induced in tumor cell killing.

The uptake kinetics and localization of the two photosensitizers were examined, using the laser scan confocal microscopy and spectrophotometric measurement. Results from spectrophotometric measurement showed that the peak fluorescence signal emitted from MC540 and mTHPC treated Neuro-2A cells was 2 hours and 16 hours, respectively. Confocal images clearly showed that the two photosensitizers were localized in the cytoplasm but not in the nucleus. Using the specific organelle probes, it was found that some of MC540 is localized in the lysosome of sensitized Neuro-2A cells. On the contrary, part of the mTHPC was found to localize at the mitochondria but not at the lysosome. The localization of mTHPC on the mitochondria might explain the effective killing of Neuro-2A cells by this compound.

Attempts were made to examine the sensitization effects of the two photosensitizers on the *in vitro* cultured rat spinal cord neurons. Maximum sensitization effect (as judged by the fluorescence intensity) was observed 15 minutes after exposure of Neuro-2A to MC540. In contrast, the uptake of mTHPC by normal rat spinal cord neurons is lower than Neuroblastoma cells.

To conclude, mTHPC was found to be more potent than MC540 on the photokilling of neuroblastoma. Both of the two photosensitizers showed a certain degree of selectivity on the sensitization effects of tumor cells versus cultured normal nerve cells.

## MC540 及 mTHPC 用於神經母細胞瘤的光敏治療

### 摘要

光敏治療是利用了一種特別的化合物，稱為光敏劑。它選擇性地對抗癌腫細胞，促使我們對它的抗癌機制引起研究興趣。

Merocyanine 540 (MC540)是一種細胞膜光敏劑，並已證實為一種治療血癌的有效光敏劑。Meta-tetrahydroxyphenyl chlorin (mTHPC)是新一代的光敏劑。它的高脂溶性及高選擇性細胞吸收使它成爲一種很有前途的光敏劑。本文比較了這兩種光敏劑對於神經母細胞瘤(Neuro-2A)的光敏療效，毒性之形成及腫瘤細胞的吸收情況作了研究。另外，這兩種光敏劑在正常的神經細胞吸收情況也作了一初步探討。

在沒有光的刺激下，MC540 及 mTHPC 均對 Neuro 2A 沒有毒性。而兩種光敏劑需要不同的時間吸收以達至最高細胞毒性。mTHPC 需要十六小時的吸收但 MC540 只需兩小時。兩種光敏劑處理過和光照射後的細胞均已失去增生的能力。

mTHPC 的光敏治療在 Neuro 2A 不能引發細胞凋亡(Apoptosis)。這可能是與對抗細胞凋亡蛋白(BCL-2)的增生有關。而 mTHPC 的光敏治療也不能導致脫氧核糖核酸(DNA)的破壞。但 mTHPC 的光敏治療卻能引發單重態氧的產生以導致神經母細胞瘤的死亡。

兩種光敏劑的吸收動力表現不同。MC540 的最高吸收在兩小時產生但 mTHPC 的最高吸收產生於十六小時。在螢光顯微鏡下，兩種光敏劑均發現只分佈於細胞質而不在細胞核。部份的 MC540 分佈於溶酶體，但在粒線體內則沒有。mTHPC 在溶酶體中沒有分佈，但部份則分佈在粒線體。

最後，兩種光敏劑在正常的老鼠脊髓細胞當中只有微量吸收，而且很快被排出細胞外。

總括而言，這兩種光敏劑均在神經母細胞瘤表現了有效的光敏治療，而在神經系統的選擇性吸收也很高。

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