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Photodynamic Effects of the Photosensitizers Zn-BC-AM and Pyropheophorbide-*a* Methyl Ester (MPPa) on Nasopharyngeal Carcinoma Cells

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Abstract

Cancer is one of the fatal diseases which claim million of life every year. The conventional therapies for cancer include chemotherapy, radiotherapy and surgery. However, there are many side effects associated with these therapeutic methods. In the past two decades, photodynamic therapy (PDT) was developed as an effective modality for the treatment of malignant diseases. During PDT, photosensitizer is preferentially absorbed and retained in the malignant tissues and then photoactivated by an appropriate wavelength of light. Subsequent photoactivation of the photosensitizer results in the tumor cell death.

In Hong Kong, Nasopharyngeal Carcinoma (NPC) is a one of the major malignant diseases. In the present study, the photodynamic activities of two photosensitizers pyropheophorbide-α methyl ester (MPPa) and a benzochlorine derivative Zn-BC-AM on the NPC cells were investigated. After irradiation at an appropriate wavelength of light, a significant cytotoxicity was observed in MPPa and Zn-BC-AM treated NPC/HONE-1 cells. The two photosensitizers killed the HONE-1 cells in a drug- and light-dose dependent manners.

The localization of the two photosensitizers in NPC cells was also examined. MPPa and Zn-BC-AM were found to localize in the mitochondria, endoplasmic reticulum (ER) and Golgi apparatus in HONE-1 cells. Results from nuclear staining and flow cytometric analysis revealed that PDT-treated NPC cells exhibited the characteristic mode of apoptotic cell death, namely DNA condensation and the appearance of sub-G1 peak. In addition to the triggering of the mitochondria-mediated apoptotic cell death pathway (i.e. induction of a rapid collapse of mitochondrial membrane potential (??m), followed by the release of cytochrome c, and activation of caspase-9 and –3) the two

sensitizers also induced ER stress in the PDT treated NPC cells. Expression of ER chaperones Bip/Grp78 and Grp94, and ER resident lectin-like chaperone calnexin (CNX) was also enhanced in PDT-stressed NPC cells. Caspase-12, an important caspase involved in ER stress-induced apoptosis, was also proleolytically activated. Inhibition of Ca^{+2} uptake into mitochondria by ruthenium red (RR) or loading the cells with EGTA-AM, an agent that buffers intracellular Ca^{+2} released from ER, resulted in a significant reduction of PDT-induced cell death. Our results indicated that ER may also play an important in the apoptotic cell death of the NPC cells. These observations suggested that both ER and mitochondria are the subcellular targets of the two sensitizers, and activation of ER- and mitochondria-mediated apoptotic pathways was responsible for MPPa and Zn-BC-AM PDT-induced NPC cell death.

Photodynamic therapy has been reported to induce the expression of cytokines, such as IL-6, IL-8, IL-10 and TNF-α in cancer cells. However, whether cytokine expression by cancer cells is directly related to the antitumor effect of PDT is unclear. It has been reported that PDT induces specific and nonspecific immune responses against tumors *in vitro* and *in vivo*. To determine whether cytokines are produced during treatment of NPC cells in Zn-BC-AM and MPPa PDT, we examined the expression of cytokines by PDT treated NPC cells. In the present studies, marked changes in the expression of cytokines profile were observed. MPPa and Zn-BC-AM PDT were found to up-regulate the mRNA expression of IL-6, IL-8, IL-12 (p35), TNF-α, MIP-1β , MPIF-1 and MPIF-2 in NPC cells. These results indicate that the PDT-induced expression of those inflammatory cytokines and chemokines may play a role in cellular sensitivity to PDT, and may be important in modulating the local antitumour immune response.

Apart from the in *vitro* studies, a NPC xenograft nude mouse model was used to evaluate the *in vivo* photodynamic efficacy of Zn-BC-AM PDT. NPC bearing nude mice were treated with 3mg/kg of Zn-BC-AM. After 24 hours, tumors were irradiated with 150 J/cm^2 (continuously or fractionated) from a diode laser (670 nm). Results from tumor response study and tumor histological examination revealed that the tumor growth was significantly reduced in PDT treated animals. Intra-tumour blood capillaries were also reduced after PDT. To conclude, our data suggest that Zn-BC-AM may be a candidate photosensitizer for the treatment of NPC.

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