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ORIGINAL ARTICLE

Meta-tetrahydroxyphenyl chlorine mediated photodynamic therapy inhibits the migration and invasion of a nasopharyngeal carcinoma KJ-1 cell line

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head and neck cancer;
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Background/Purpose: Different photosensitizer-mediated photodynamic therapy (PDT) has different intracellular cytotoxic cascades. Previous reports showed that 5-aminolevulinic acid (5-ALA)-mediated PDT suppressed the migration and invasion of head and neck cancer cells. Unlike from 5-ALA, which mainly targets the mitochondria of cells, meta-tetrahydroxyphenyl chlorin (m-THPC) mainly accumulates in the endoplasmic reticulum and Golgi complex. Does m-THPC PDT inhibit the migration and invasion of cancer cells?
Methods: The effect of m-THPC PDT with a sublethal dose sufficient to kill around 20% of cells on the migration and invasion of a nasopharyngeal carcinoma KJ-1 cell line was studied by wound healing and Matrigel invasion assays.
Results: In the wound healing assay, the migration distance of KJ-1 cells decreased significantly from 0.71 $\pm$ 0.02 mm in the control cells to 0.31 $\pm$ 0.03 mm in the PDT-treated cells 24 hours after light treatment ($p < 0.05$) and the migration distance also decreased significantly from 1.02 $\pm$ 0.07 mm in the control cells to 0.32 $\pm$ 0.04 mm in the PDT-treated cells 48 hours after treatment ($p < 0.05$). In the Matrigel invasion assay, the number of the invaded KJ-1 cells in PDT treated group was also statistically significantly less than those without PDT treatment ($p < 0.05$).

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Conclusion: This study demonstrates that a sublethal dose of m-THPC PDT inhibits the migration and invasion of nasopharyngeal carcinoma cells *in vitro*.

Introduction

Photodynamic therapy (PDT) involves a photosensitizer, light, and oxygen. After administration of the photosensitizer for a given period to allow the drug selectively accumulated in the target tissue, the photosensitizer is activated by nonthermal light of a wavelength matched to its absorption characteristics and then transfers energy from light to oxygen, to generate reactive oxygen species (ROS), which damage the intracellular structure and cause cell death.[1] Due to good tumor response, better anatomical and functional preservations of normal tissue and no cumulative toxicity, PDT has been approved to be an alternative treatment modality of cancers in addition to surgery, radiation, and chemotherapy.[1,2]

As far as we know, the process of cell migration and invasion involves dissolution of the extracellular matrix surrounding the cells, cell protrusion and attachment of the leading edge, cell contraction by myosin motors inserting between actin bundles, and detachment of the adhesions at the trailing edge of the cell.[3] This process plays a very important role in cancer metastasis, which involves: migration of cancer cells into the blood vessels; survival of cancer cells in the systemic circulation and transport to target organs; arrest of cancer cells in the microcirculation; migration of cancer cells through the vessels wall into the interstitial space; and then proliferation of cancer cells in the target organs.[4]All of these have complex biological mechanisms, which include many chemotaxis mediators, cell-matrix chemical ligand-receptor interactions and intracellular signaling network.

One previous study reported the experience of treating 45 locally recurrent head and neck cancer patients with PDT. Among them, 15 patients received more than one PDT treatment and none had developed distant metastasis during the follow-up period.[5] Thus, anecdotally, PDT seems to have the potential of inhibiting the invasiveness and metastasis of cancer cells. Therefore, we previously studied the effect of 5-aminolevulinic acid (5-ALA)-mediated PDT on the migration and invasion of head and neck cancer cell lines. The study showed that 5-ALA PDT suppressed the migration and invasion of head and neck cancer cells, probably due to inhibition of tyrosine phosphorylation of the focal adhesion kinase (FAK) and its down-stream Src kinase-extracellular signal-regulated kinase (ERK) signaling pathway.[6]

Because different photosensitizers mainly accumulate in different subcellular organelles, PDT will initially attack these organelles, which might initiate different intracellular cytotoxic cascades.[1,7] 5-ALA is converted into protoporphyrin IX (PpIX) within the mitochondria of cells, which is a potent endogenous photosensitizer.[7] Therefore, 5-ALA PDT initially damages the mitochondria, which provokes cell apoptosis and necrosis. Unlike 5-ALA, meta-tetrahydroxyphenyl chlorin (m-THPC, Foscan), which is a potent second-generation photosensitizer and has been approved using in palliative treatment for head and neck cancers, mainly accumulates in the endoplasmic reticulum and Golgi complex.[1,7–9] Does m-THPC PDT also inhibit the migration and invasion abilities of cancer cells? In this paper, we studied the effect of m-THPC PDT on the migration and invasion of a nasopharyngeal carcinoma cell line by use of wound healing and Matrigel invasion assays. Our results showed that m-THPC PDT also inhibited the migration and invasion of nasopharyngeal carcinoma cancer cells.

Materials and methods

Cell culture

Human nasopharyngeal undifferentiated carcinoma cell line KJ-1 was used. Stock cultures were grown in Dulbecco's modified Eagle's medium supplemented with 2 mM L-glutamine, 1% penicillin/streptomycin solution, and 10% fetal calf serum (Life Technologies Inc., Paisley, UK). Cell cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

PDT

For PDT, the cells were incubated with 0.2 μg/ml of m-THPC (Biolitec Pharmaceuticals Ltd, Jena, Germany) for 24 hours, washed thoroughly in PBS, and then exposed to various doses of light. The light source for m-THPC activation was a diode laser with an emission of red light at a wavelength of 652 nm (fluence rate: 100 mW/cm^2, Biolitec). After light treatment, the cells were incubated with fresh complete medium for further experiments. The light doses used in the wound healing and Matrigel invasion assays were sufficient to kill 20% of the cells. The control group consisted of the cells that were incubated with the photosensitizer but without light treatment. The results were obtained from at least three individual experiments.

Assessment of photosensitizer-induced cytotoxicity

The cell viability was determined by using the 3(4,5-dimethyl-thiazoyl-2-yl) 2,5 diphenyl-tetrazolium bromide (MTT) assay 24 hours after light treatment. In brief, cells were incubated with MTT solution for 3 hours. Then, MTT solution was removed and DMSO added. Optical readings of absorbance were performed at 570 nm using a microplate reader. Control groups consisted of cells that were not incubated with the photosensitizer and had no light treatment, and cells that were incubated with the

photosensitizer but without light treatment. The results were obtained from at least three individual experiments.

Wound healing assay

The cells were grown to confluence in 3.5 cm petri dishes and then subjected to PDT. After PDT, the cell monolayer was lesioned using a 1.2 mm cell scraper without damaging the dish surface. Lesion areas were imaged at 0, 24, and 48 hours after PDT. The photos were analyzed with imaging software. The distance of cell migration was calculated by subtracting the distance between the lesion edges at 24 and 48 hours from the distance measured at 0 hour. The values were expressed in millimeters.

Matrigel invasion assay

The invasiveness of cells before and after PDT was measured by using the Matrigel invasion assay as described previously.[6] Briefly, 24-transwell inserts with 8-μm pores (Becton Dickinson, Franklin Lakes, NJ, USA) were coated with Matrigel (0.77 g/l; Becton Dickinson, Bedford, MA, USA). The PDT-treated and non-treated cancer cells were detached by trypsinization, washed with complete medium and resuspended in serum free DMEM 24 hours after light treatment. Then, cells at a density of 1×10^4 were seeded in the upper chambers with 150 μl of medium. Medium supplemented with 10% FCS was placed in the lower wells. After 24 hours and 48 hours, cells that had invaded to the lower surface of the Matrigel-coated membrane were fixed with 70% ethanol, stained with hematoxylin, and counted under a light microscope. For each experimental condition, the assay was performed at least in triplicate.

Statistical analysis

All statistical analyses were performed using GraphPad prism 3.02 (GraphPad Software, San Diego, CA). The Student's *t* tests were used to determine the differences of the migration distances and the numbers of the invaded cells between PDT treated and nontreated groups. Corresponding *p*-values <0.05 were interpreted as statistically significant.

Results

Photodynamic treatment

The cytotoxic effect of m-THPC PDT on the cells was shown in Fig. 1. The nasopharyngeal carcinoma KJ-1 cells were very sensitive to m-THPC PDT. The light dose that caused 50% of cell death was around 0.7 J/cm^2.

m-THPC PDT suppressed the migration and invasion of KJ-1 cells

To evaluate the effect of m-THPC PDT on the migration and invasion of KJ-1 cancer cells, a sublethal dose that killed only 20% of the cells was used. The light dose in this study was 0.3 J/cm^2. In the wound healing assay, m-THPC PDT

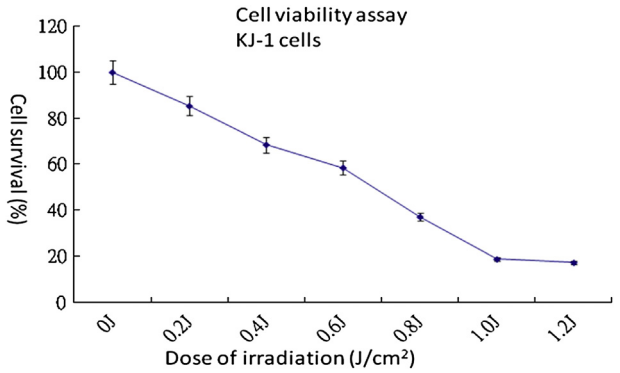

Figure 1 Cytotoxicity of m-THPC PDT on nasopharyngeal carcinoma KJ-1 cells. KJ-1 cells were incubated with 0.2 μg/ml of m-THPC for 24 hours and then exposed to light irradiation at different light doses. Cell viability was assessed by MTT assay 24 hours after light irradiation. Data were obtained from three experiments. Bars, SE.

significantly suppressed the migration of KJ-1 cells 24 hours and 48 hours after light treatments (Fig. 2). The migration distance of KJ-1 cells decreased significantly from 0.71 ± 0.02 mm in the control cells to 0.31 ± 0.03 mm in the PDT-treated cells 24 hours after light treatment ($p < 0.05$). Forty-eight hours after light treatment, the migration distance of cells also decreased significantly from 1.02 ± 0.07 mm in the control cells to 0.32 ± 0.04 mm in the PDT-treated cells ($p < 0.05$). In the Matrigel invasion assay, the numbers of the invaded KJ-1 cells in PDT treated groups, either 24 hours or 48 hours after cells were seeded in the upper chamber, were statistically significantly less than those without PDT treatment (24 hour: $p = 0.03$, 48 hour: $p = 0.01$), which mean m-THPC PDT also suppressed the invasiveness of KJ-1 cells (Fig. 3).

Discussion

Head and neck cancer is known to be locally aggressive and highly invasive. It has a tendency to invade the surround tissue and metastasize to regional lymphatics or distant vital organs. The main treatment for head and neck cancers includes surgery, radiotherapy, and chemotherapy. However, surgery and radiotherapy might contribute to wide spreading of cancer cells if incomplete resection and ablation of the tumor. This may be due to destruction of local natural barriers, change of tumor microenvironment and enhancement of invasiveness of cancer cells.[10] On the contrary to the conventional treatments, PDT might have the potential of inhibiting the invasiveness and metastasis of incompletely treated head and neck cancer cells clinically.[5,11] This anti-invasion/metastasis effect may be due to enhancement of anti-tumor immunity and inhibition of cancer cells *per se* by PDT.[6,12] Lisnjak et al showed that 5-ALA PDT decreased the angiogenesis and metastasis of cancer cells in a mice Lewis lung carcinoma model.[13] Our previous study also showed that a sublethal dose of 5-ALA PDT significantly suppressed the migration and invasion of head and neck cancer cells *in vitro*,[6] and inhibited the metastatic ability of cancer cells in chick embryo chorioallantoic membrane model (unpublished data). We also

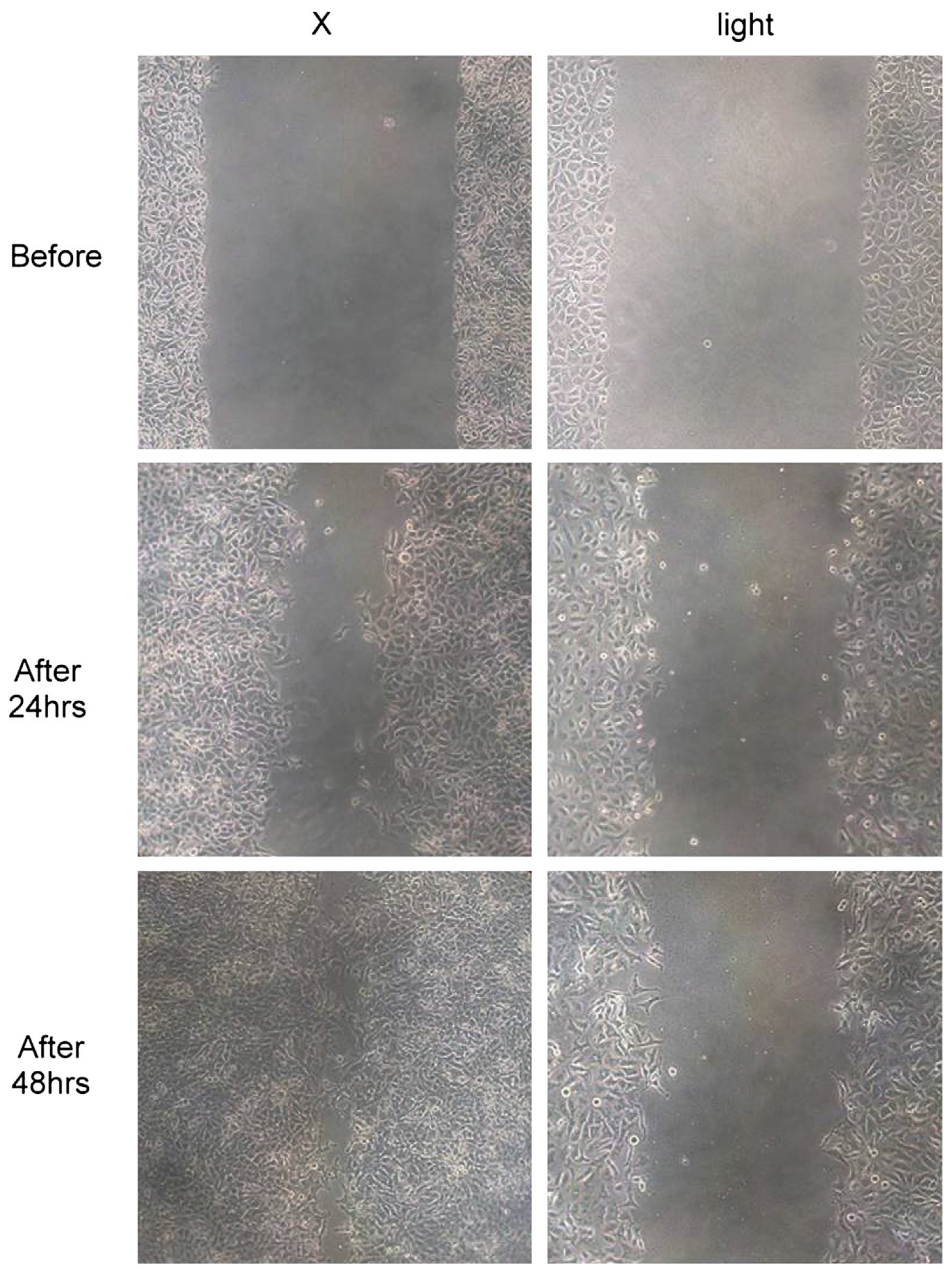

Figure 2 m-THPC PDT suppressed cell migration in wound healing assay. Monolayer of KJ-1 cells was lesioned by a scraper in petri dish. Repair of lesion by cell migration with or without PDT was photographed 24 hours and 48 hours later.

demonstrated that 5-ALA PDT also inhibited tyrosine phosphorylation of the focal adhesion kinase and its downstream Src kinase-extracellular signal-regulated kinase signaling pathway, which serves an important regulator of cell migration.[6,14] A more recent study by Tsai et al also showed that 5-ALA PDT could reduce epidermal growth factor receptor expression and invasion of the surviving cancer cells and these effects could further pass to the progeny *in vitro*.[15]

Because singlet oxygen, the main cytotoxic product during PDT, has a life-time <0.05 μs in cells with a diffuse length of <0.02 μm, PDT of different photosensitizers initiates different intracellular cytotoxic cascades. Despite these differences, photosensitizer-mediated PDT has similar antitumor gross effects, including direct cytotoxicity, shutdown of tumor-associated vasculature, and activation of antitumor immunity.[1,7] For example, aluminum phthalocyanine disulfonate and chloroaluminum tetrasulfonated phthalocyanine (CASPc), which enter the cell via endocytosis and accumulate in the membrane of or within the endosome/lysosomes of cells,[7,16] have an additional ability to rupture the endosome//lysosomes so that other cytotoxic drugs within the endosome/lysosomes can be released into the cytoplasm of cells (photochemical internalization, PCI effect).[17] Other photosensitizers, such as 5-ALA, which mainly accumulate in the motochondria of cells and which are in the endoplasmic reticulum and Golgi complex like m-THPC, have no such PCI effect after light treatment.

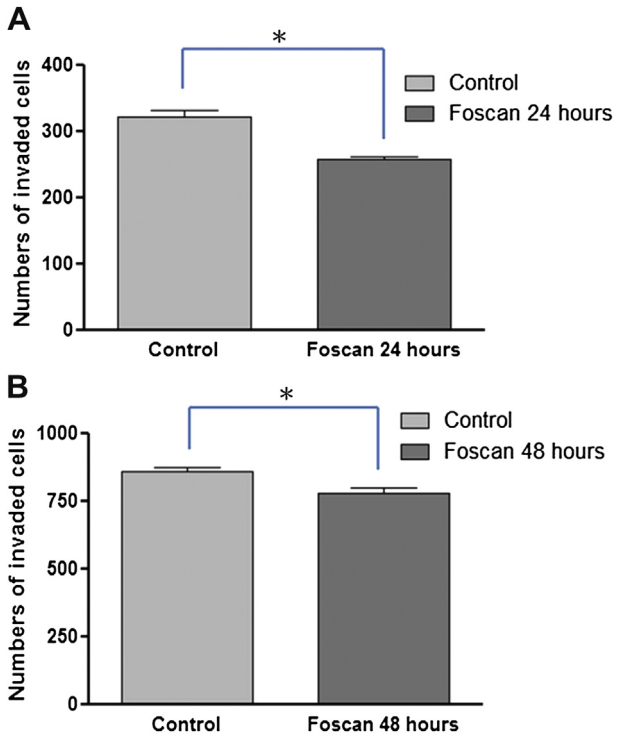

Figure 3 Matrigel invasion assay. The cell numbers of the invaded cells were compared between the control group and PDT group, (A) 24 hours and (B) 48 hours after the KJ-1 cells were seeded in the upper chambers (24 hour: $p = 0.03$, 48 hour: $p = 0.01$). Data were obtained from three independent experiments. *$p < 0.05$.

Regarding the studies on the effect of PDT of different photosensitizers on cell migration and invasion other than 5-ALA, Rousset et al reported that hematoporphyrin derivative (HPD)-PDT decreased cancer cell adhesiveness to endothelial cells *in vitro* and reduced the metastatic potential of cells injected into rats[18]; and Waterman et al showed that CASPc PDT inhibited the migration of cultured human vascular cells via acting on secreted and extracellular matrix proteins *in vitro*.[19] Sharwani et al demonstrated that m-THPC PDT suppressed the activities of matrix metalloproteinase-2, -9, and -13, urokinase plasminogen activator and vascular endothelial growth factor in three oral cancer cell lines; and suggested the inhibition effect of m-THPC PDT on tumor invasiveness.[20] However, Sharwani's study did not show the effect of m-THPC PDT on the migration and invasion of cancer cells. Our observation in this study could support Sharwani's suggestion and showed that m-THPC PDT may be able to inhibit the migration and invasion of head and neck cancer cells, similar to the effect of ALA-PDT. Despite only a nasopharyngeal carcinoma cancer cell being evaluated in this study, combined with all studies previously reported, our data imply that the inhibition of PDT on cancer cell migration and invasion may be a important treatment effect among different photosensitizers in different cancer cells. This effect may be considered for clinical use but it needs to be elucidated further.

In conclusion, this study demonstrates that a sublethal dose of m-THPC PDT also inhibits the migration and invasion of head and neck cancer cells despite m-THPC have different subcellular localization from 5-ALA.

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