Antiproliferative and cytotoxicity effects of aluminium (iii) phthalocyanine chloride tetra sulphonic acid-mediated photodynamic therapy on oesophageal cancer

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Abstract. Oesophageal cancer is an aggressive and lethal malignancy accounting for the eighth leading cause of cancer and sixth cause of cancer-related death globally. Conventional treatments for oesophageal cancer are characterised by suboptimal efficiency resulting in treatment resistance and relapse. Photodynamic therapy (PDT), a non-invasive modality, has emerged as a potential alternative cancer therapy. Growing evidence has shown that aluminium (III) Phthalocyanine Chloride Tetra sulfonic Acid ($AlPcS_4Cl$) is a promising photosensitiser in PDT owing to its photochemical and photophysical features. This study examined the antiproliferative and cytotoxic impacts of AlPcS4Cl-mediated PDT in an oesophageal cancer cell line (HKESC-1). The HKESC-1 cells were grown and maintained in a culture medium incubated at 37° C, with 5% CO2 and 85% humidity. The cells were treated with increasing dose concentrations of AlPcS4Cl and irradiated at a fluence of 5 J/cm2 using a diode laser at 673.2nm wavelength. The cellular activities following 24 hours post-PDT were evaluated using microscopy and biochemical tests to determine the response of HKESC-1 cells to treatments. Results from treated cells displayed a dose-dependent response as shown by the significant morphologic changes, increased cytotoxic damage, and reduced cell viability and proliferation. Fluorescent microscopy revealed that AlPcS4Cl was internalised in the mitochondria and lysosomes, suggesting the possible cell death pathways. The study showed that AlPcS4Cl mediate PDT is an efficient treatment modality for oesophageal cancer. Further research on the mechanism of cell death pathways in oesophageal cancer could enhance and translate the potential application of AlPcS4Cl mediated PDT of cancer in clinical settings.

1. Introduction

Oesophageal cancer is an aggressive and lethal malignancy accounting for the eighth leading cause of cancer and the sixth cause of cancer-related death worldwide [1]. Conventional treatments for oesophageal cancer consist of surgery, chemotherapy, and radiotherapy [2], and recently immunotherapy has also been employed for the treatment of oesophageal cancer [2, 3]. However, these conventional therapies are characterised by suboptimal efficiency resulting in treatment resistance, relapse, and severe adverse effects. Also, not all patients are eligible for immunotherapy. Photodynamic therapy (PDT), a non-invasive modality, has emerged as a potential alternative cancer therapy for either curative or palliative purposes. PDT has gained much attention due to its effectiveness, limited toxicity, compatibility with other treatments, can be repeated several times, and cost-effectiveness [4].

PDT is made up of three crucial components, photosensitiser (PS), light and oxygen. The PS, a light-activated agent, is first administered and internalised within the tumour cells, then irradiated with

suitable light at a particular wavelength, resulting in reactive oxygen species (ROS) generation, oxidative stress, damage to cellular organelles /membranes and ultimately the death of cancer cells [5]. Growing evidence indicates that Aluminium (III) Phthalocyanine Chloride Tetra Sulfonic Acid ($AlPcS_4Cl$) is a promising photosensitiser in PDT for various tumours due to its distinct photochemical and photophysical features [6]. However, its cellular impacts on HKESC-1 oesophageal cancer cells are limited. This study examined the antiproliferative and cytotoxic impacts of AlPcS4Cl-mediated PDT in an oesophageal cancer cell line (HKESC-1).

2. Method

2.1. Cell culture

A human oesophageal cancer cell line (HKESC-1) used in this study was purchased from Cellonex (South Africa), a distributor of AddexBio. HKESC-1 cells were maintained in T75 culture flasks in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, D5796) with the addition of 10% Fetal Bovine Serum (FBS) (Biochrom, S0615), 1mM sodium pyruvate 1% antibiotic: 0.5% Amphotericin B and 0.5% Penicillin-streptomycin. The cells were maintained under 5% CO_2, 37°C and 80% humidity.

2.2. Photodynamic Treatment

Experimental and control cells were cultured with a seeding density of 5 x 10^5 in culture Petri dishes of 3.4 cm^2 diameter until cells attain 90 percent confluence. The cells then received different concentrations of the PS ($AlPcS_4Cl$) (1.25, 2.5, 5, 10 & 20µM) and were incubated for four hours at 37˚C and 5% CO_2 to allow cellular localisation of the PSs. After incubation, the cells were subjected to irradiation using a continuous semiconductor diode laser (Oriel Corporation) at 673 nm and 5 J/cm^2 fluency. After that, the irradiated culture plates were incubated for 24 hours before biochemical assays were conducted.

2.3. Cell Morphology

Cellular morphological changes were visualised and examined with an inverted light microscope (Wirsam, Olympus CKX41Q4), and images taken with an Olympus digital camera.

2.4. Cellular Proliferation Assay

Cellular proliferation examination was conducted using the CellTiter-Glo® 3D luminescence reagent (Promega, G968A) to determine the level of ATP in live cells. Briefly, 50µL cell suspension and 50µL of the reconstituted reagent were added into an opaque-walled 96 well plate and carefully mixed. The plate was placed on a shaker for 2 minutes to facilitate lysis and kept in the dark at ambient temperature for 10 minutes. The ATP luminescence generated was quantified using the PerkinElmer, VICTOR Nivo™.

2.5. Viability

Cell viability assay was performed using the Trypan blue exclusion test to determine the proportion of live cells. Ten microlitres of 0.4% Trypan blue (Sigma Aldrich, T8154) were added to an equivalent volume of cell suspension and mixed properly. Then, the 10µL volume was pipetted to a counting chamber slide and slotted into an automated cell counter (Countess®).

2.6. Cytotoxicity

A cytotoxicity assay was conducted to measure the lactate dehydrogenase (LDH) enzyme released by the damaged cell membrane. The test was achieved using the Cyto Tox 96® Non-Radioactive Cytotoxicity assay (Promega, G179A). Briefly, 50µL of reconstituted substrate mix and 50µL of cell suspension were added into a 96 multi-well plate and mixed. The plate was wrapped with foil and kept in the dark for 30 minutes at 25°C. The colour produced was measured using PerkinElmer, VICTOR Nivo™ at an absorbance of 490nm.

2.7. Data Analysis

All results collated and analysed using GraphPad Prism (v5). All experiments were conducted at least two times and in biological triplicates (n=3). The mean values of test groups were evaluated in relation with the mean value of control cells. One-way ANOVA was employed, and a statistical significance was defined as p <0.05 (*), p <0.01 (**), and p <0.001 (***).

3. Result and Discussion

3.1. Cellular Morphology

We studied the effect of $AlPcS_4Cl$-PDT on the morphology of HKESC-1 cells by examining the cellular alterations after incubation with varying concentrations of $AlPcS_4Cl$ (1.25-20 µM) pre and post irradiation. Our findings from cells without irradiation showed that $AlPcS_4Cl$ do not have a cytotoxic effect on HKESC-1 cells compared to the untreated control (Figure 1A). No morphological changes were observed in the absence of light. No differences were seen between the control cells with no PS and cells with PS. However, the irradiated cells with $AlPcS_4Cl$ at 5J/cm^2 showed significant cellular morphological changes (Figure 1B). Morphological impairments showed 24 hours after treatment was shrinkage of cells, membrane integrity distortion, and floatation of cells in culture plates. The morphological changes in the treated irradiated group were indicative of cell death.

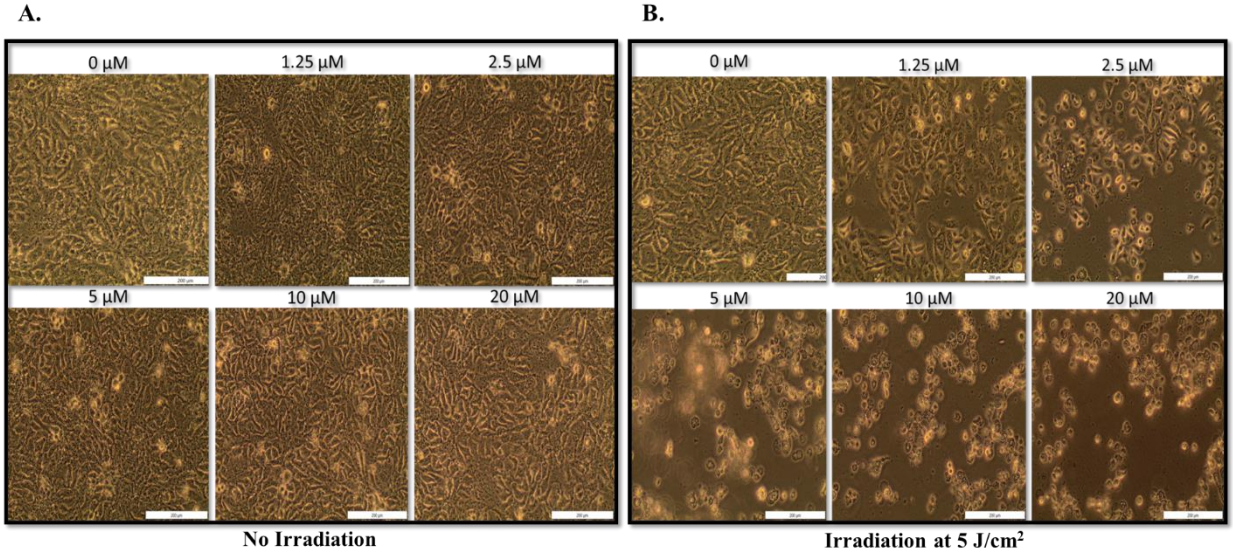

Figure 1. Cellular morphological changes in HKESC-1 cells 24 hours post treatments. (A) The morphological changes in the presence of different concentrations of $AlPcS_4Cl$ (without irradiation). (B) The morphological changes of $AlPcS_4Cl$-PDT at 5 J/cm^2 irradiation.

3.2. Cellular Proliferation Assay

We evaluated the antiproliferative effects of $AlPcS_4Cl$-PDT on HKESC-1 cells by measuring the ATP levels, which is a function of cell viability and active cellular proliferative strength. Damage cell membrane results in the ability to produce ATP, and the intracellular ATPases rapidly diminish the ATP in the cytoplasm [7]. The ATP levels were quantified using CellTiter-Glo® 3D luminous assay after incubation with varying concentrations of $AlPcS_4Cl$ (1.25-20 µM) without irradiation and with irradiation. Our results revealed high ATP levels in non-irradiated cells and active proliferative capabilities both in the control cells and the cell receiving $AlPcS_4Cl$ (Figure 2A). The findings showed that the PS does not affect the proliferation rate of the cancer cells when in an un-activated state.

In contrast, the combination of $AlPcS_4Cl$ with irradiation at 5 J/Cm^2 significantly showed a dose-dependent reduction of ATP levels and high antiproliferative effects on HKESC-1 cells (***p< 0.001) when compared with the control cells (Figure 2B). This finding is in concordance with the results from

Crous and coworkers, who investigated the effect of AlPcS$_4$Cl-PDT on lung cancer [6] and Kresfelder et al. (2009) who examined the effects of AlPcSmix on oesophageal cancer [8].

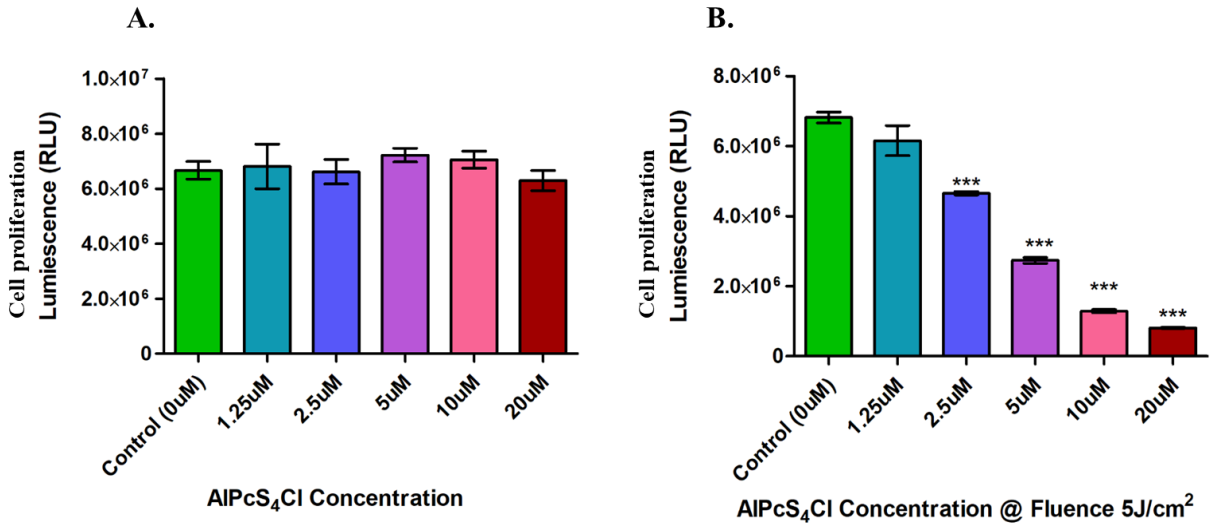

Figure 2. The Antiproliferative effects of AlPcS$_4$Cl on HKESC-1 cells. (A) Non-irradiated cells showed high levels of ATP and active proliferation with no significant difference between the control cells and cells administered with AlPcS$_4$Cl. (B) The irradiated cells at 5 J/cm^2 displayed significant antiproliferative activities with a reduction in ATP levels (***$p < 0.001$). The values shown are ± SEM (standard error of the mean) (n=3).

3.3. Viability

Trypan blue exclusion test was conducted to determine the proportion of live cells present after 24-hour administration of AlPcS$_4$Cl and AlPcS$_4$Cl-PDT on HKESC-1 cells. Trypan blue exclusion test is based on the mechanism that the cell membrane integrity of viable cells is intact and therefore expels the Trypan blue dye. In contrast, dead cells retain the stain due to damaged cell membrane [7, 9]. The proportion of live cells was measured using an automated cell counter. The result showed that cells treated with different concentrations of AlPcS$_4$Cl without irradiation had a high percentage of viable cells which was similar to the untreated control cells (Figure 3A). The findings showed that the PS does not inhibit cell viability in the cancer cells without irradiation. However, substantial differences were seen between the control and the PDT groups, as depicted in Figure 3B (*$p < 0.05$, ***$p < 0.001$). The results demonstrated that the PS substantially decreased cell viability rate post-PDT. Comparable findings have been reported on aluminium phthalocyanines based PS on various cancers such as oesophageal cancer [8], lung cancer [6], breast cancer [10], and cervical cancer [11].

3.4. Cytotoxicity

Cytotoxicity assay using lactate dehydrogenase (LDH) release method was employed quantify the amount of LDH enzyme leakage in the cell culture media. Cells that have damaged cell membrane release LDH, which indicates cytotoxic effects [7, 12]. We found that no statistically significant were observed in the non-irradiated (between the control cells and the cells administered with PS) (Figure 4A). Although the non-irradiated cells showed some slight cytotoxic effect compared with the positive control, a common feature of tumour cells undergoing constant proliferation, and so experiencing minor cell death. This observation is consistent with findings from Robertson and colleagues, who examined the cytotoxic effect of metallophthalocyanine (MPc) on human metastatic melanoma cells [13]. The LDH released from damaged cells from the irradiated cells administered with 0–20 μM of PS at 5 J/cm^2 were compared to the control receiving no PS and cytotoxicity positive control. The AlPcS$_4$Cl-PDT induced significant cytotoxicity compared to the control receiving no PS (*$p < 0.05$, **$p < 0.01$) (Figure 4B), which is also in agreement with a study reported by Crous et al. (2019) who investigated the

cytotoxic effect of this PS on lung cancer[6]. In addition, similar results were shown by studies that examined the cytotoxic of AlPcSmix on oesophageal cancer cells [8] and cervical cancer[11].

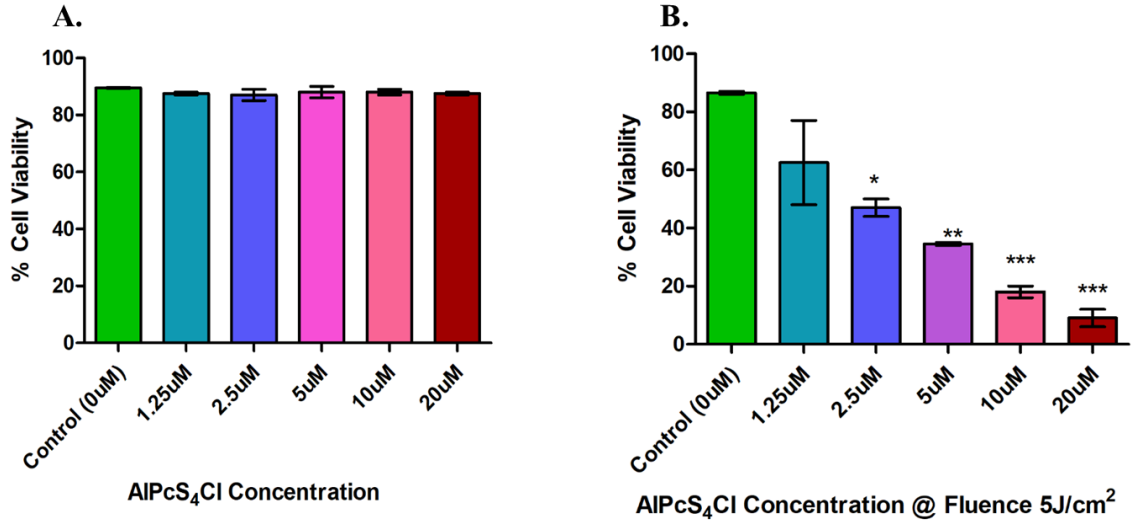

Figure 3. Fig. 1. Cell viability effect of $AlPcS_4Cl$ ON HKESC-1 using Trypan blue exclusion assay. (A) The percentage of cell viability of non-irradiated cells in different concentrations of $AlPcS_4Cl$. (B) The percentage of cell viability irradiated with 5 J/cm^2 after $AlPcS_4Cl$ -PDT treatment. The findings are shown as ± SEM (n = 3); (*p < 0.05, **p < 0.01, ***p < 0.001).

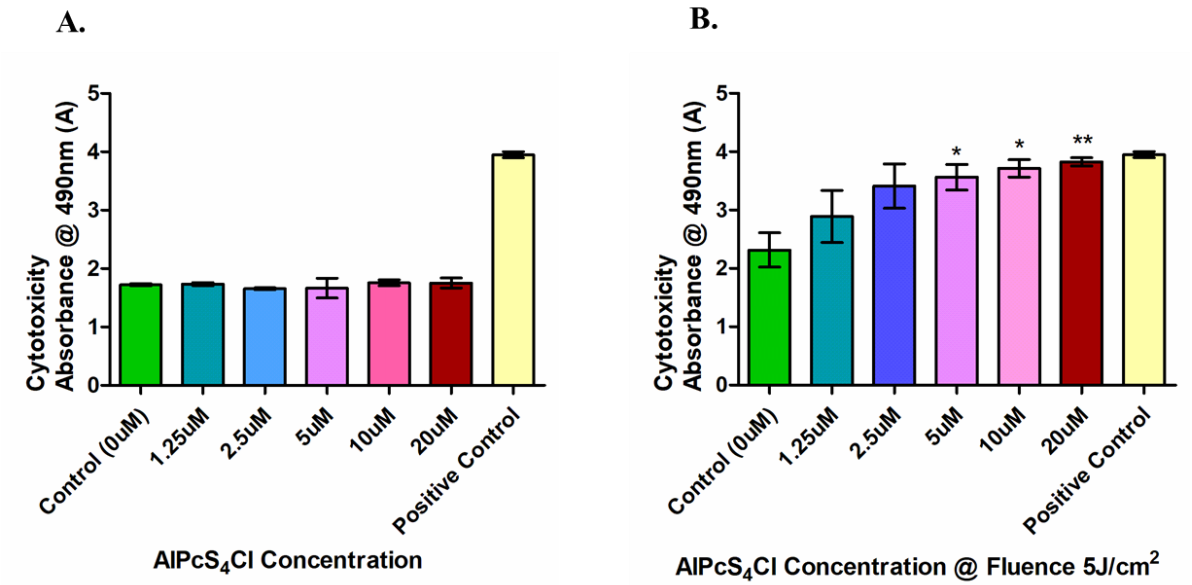

Figure 4. The cytotoxic effects of $AlPcS_4Cl$ and $AlPcS_4Cl$-PDT were determined 24 hours after treatments by LDH release cytotoxicity assay on HKESC-1 cells. (A) The levels of LDH leakage in the presence of varying concentrations of $AlPcS_4Cl$ (non-irradiation). (B) The levels of LDH release after $AlPcS_4Cl$-PDT with 5 J/cm^2 irradiation. The results are depicted as ± SEM (n = 3); (*p < 0.05, **p < 0.01).

4. Conclusion

Photodynamic therapy, a non-invasive and non-toxic treatment modality, has emerged as an effective alternative treatment modality required to overcome the challenges associated with the current conventional therapy for various malignancies. Aluminium phthalocyanine and its derivatives have been

investigated in different tumours with promising outcomes. The findings from this study revealed that $AlPcS_4Cl$ mediated PDT can significantly reduce cell viability, inhibit cell proliferation, and have cytotoxic effects on oesophageal cancer. Also, the result showed a negligible dark-cytotoxicity activity on oesophageal cancer, therefore $AlPcS_4Cl$-PDT offers a promising treatment modality for oesophageal cancer. Further investigation is needed to determine the cell death mechanism and pathways.

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