*Polish J. of Environ. Stud. Vol. 15 No. 4A (2006), 163-165*

# Effect of Photodynamic Therapy with 5-Aminolevulinic Acid on Bladder Cancer Cells

**E. Szliszka[1], Z. P. Czuba[1], B. Beck[2], A. Sieron[3], W. Krol[1]**

[1]Chair and Department of Microbiology and Immunology,
[2]Chair and Department of Biophysics, ul. Jordana 19, 41 808 Zabrze
[3]Chair and Clinical Department of Internal Diseases and Physical Medicine, Center for Laser Diagnostics and Therapy, ul. Batorego 15, 41 902 Bytom, Medical University of Silesia, Katowice, Poland

## Abstract

Bladder cancer accounts for approximately 5% of all cancer deaths in humans. The large majorities of bladder tumors are superficial at diagnosis and, after local surgical therapy, have a high rate of local recurrence and progression. Current treatments extend time to recurrence but not alter survival. Photodynamic therapy (PDT) is an accepted therapeutic procedure suitable for the treatment of a variety of tumors, particularly against superficial bladder cancer. In this study, we investigated the effect of ALA-mediated PDT on human bladder transitional cancer cell (TCC) lines. Cytotoxicity was determined by MTT and LDH assays. The results show that ALA-induced photosensitization has a high potential for PDT of transitional bladder cancer.

**Keywords**: PDT, ALA, bladder cancer

## Introduction

Bladder cancer accounts for approximately 5% of all cancer deaths in humans. It consists mainly of superficial transitional carcinomas (TCC) and is characterized by frequent recurrence and risk to progress toward invasive disease [1]. Therefore, there is a need to develop novel treatment strategies that selectively induce cytotoxicity of bladder cancer cells. Photodynamic therapy (PDT) is an approved anticancer therapy that kills cancer cells by the photochemical generation of reactive oxygen species following absorption of visible light by a photosensitizer, which selectively accumulates in tumors [2,3]. Bladder cancer is an ideal tumor for photodynamic therapy because endoscopic access is convenient and usually a superficial, multifocal disease allows for good light penetration [4].

## Materials and methods

Human bladder cancer cell lines. The TCC cell lines used in this study display phenotypes associated with well differentiated – SW780, moderately differentiated – 647V, poorly differentiated – T24. The bladder cells SW780 were purchased from the ATCC (Manassas, VA, USA), but 647V and T24 were obtained from DSMZ (Braunschweig, Germany). Photosensitizer. 5-aminolaevulinic acid (Calbiochem, San Diego, CA, USA).

*In vitro PDT***.** Stock solutions of 5-aminolaevulinic acid (ALA) were prepared in deionized water. Before incubation of cells further dilutions were made with medium to obtain final concentrations as indicated. Bladder cancer cell lines were seeded into 96-well plates at the concentrations of 2 x $10^4$ cells of each cell lines per well and grown for 24 hours.

Under low light conditions the cells were incubated with ALA at the final concentrations of 5, 10, 25 and 50 μM for 4 hours, the medium was removed and the cells were washed twice with PBS. The cells were then irradiated with VIS (400 – 750 nm, 7.5 J/cm$^2$ and 15 J/cm$^2$) delivered from the incoherent light source PDT TP-1 (Cosmedico Medizintechnik GmbH, Schwenningen, Germany). The cytotoxic effect of PDT on human bladder cell lines was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [5] and lactate dehydrogenase (LDH) tests [6] as previously described.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. After PDT culture medium was removed, TCC cells were incubated for 24 hours as indicated. Controls included native cells and medium alone. Following incubation 20 μl of MTT (5 mg/ml) (Sigma-Aldrich, St. Louis, MO) was added per well and cells cultures incubation was continued for the next 4 hours at 37 $^o$C. The formazan crystals were dissolved in DMSO. The spectrophotometric absorbance of each well was measured by microculture plate reader with the test wavelength of 550 nm. The percentage of cytotoxicity was calculated by the formula: percentage cytotoxicity = (1- [absorbance of experimental wells/absorbance of control wells]) x 100.

LDH (lactate dehydrogenase) release assay. LDH is a stable cytosolic enzyme, which is released upon membrane damage. LDH activity was measured using a commercial cytotoxicity assay kit (Roche Molecular Biochemicals, Germany), in which released LDH in culture supernatants is measured with a coupled enzymatic assay, resulting in conversion of a tetrazolium salt into red formazan product. The cells were treated with PDT an indicated period time. The sample solution (supernatant) was removed and LDH released from cells was measured in culture medium. Maximal release was obtained after treating control cells with 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO). The necrotic percentage was expressed using the formula: (sample value/maximal release) x 100%.

## Results

Cytototoxicity against bladder cancer cells after treatment with PDT. We investigated the susceptibility to PDT induces in transitional bladder cancer cells using MTT. The photocytotoxicity showed an ALA- and ligh-dose dependent increase (Table 1). The 7.5 J/cm$^2$ light photoactivated ALA at the concentration of 50 μM in all TCC lines showed similar cytototoxic results (SW780 – 21.3±5.2%, 647V – 21.8±3.0%, T24 – 28.8±0.5% ). The TCC cells treated with 15 J/cm$^2$ light and ALA at the same concentrations showed significantly higher cytotoxicity (SW780 – 89.2±2.9%, 647V – 92.3±0.5 and T24 – 92.7±0.7% respectively). ALA and light alone had no effect on bladder cancer cells. The necrotic cell death percentage of all bladder cancer cell lines incubated with ALA, treated with 7.5 J/cm$^2$ light and examined by LDH release test was almost near zero and increased significantly with 15 J/cm$^2$ light dose.

**Table 1.** Cytotoxic effect of ALA-mediated PDT on the transitional bladder cancer cell lines: SW780, 647V and T24. The TCC lines were treated with PDT (VIS 400 – 750 nm, 7.5 J/cm$^2$ and 15 J/cm$^2$, ALA at the concentrations of 5 – 50 μM). Twenty four hours later the photocytotoxicity was meaasured by MTT.

| **Light energy 7.5 J/cm$^2$** | | | |
|---|---|---|---|
| **ALA [μM]** | **Photocytotoxicity [%] [a]** | | |
| | **SW780** | **647V** | **T24** |
| 0 | 1.6 ± 1.0 | 0,9 ± 0,9 | 1.6 ± 1.0 |
| 5 | 8.8 ± 3.6 | 6.2 ± 1.7 | 6.5 ± 2.1 |
| 10 | 14.5 ± 2.1 | 8.4 ± 0.4 | 15.5 ± 0,5 |
| 25 | 16.5 ± 1.0 | 11.6 ± 1.7 | 17.5 ± 0.6 |
| 50 | 21.3 ± 5.2 | 21.8± 3.0 | 28.8 ± 0.5 |
| **Light energy 15 J/cm$^2$** | | | |
| **ALA [μM]** | **Photocytotoxicity [%] [a]** | | |
| | **SW780** | **647V** | **T24** |
| 0 | 0.8 ± 0.7 | 0.9 ± 0.9 | 1.3 ± 1.4 |
| 5 | 31.1 ± 3.0 | 9.4 ± 1.8 | 8.5 ± 0.4 |
| 10 | 41.5 ± 4.0 | 25.2 ± 1.5 | 37.8 ± 0.5 |
| 25 | 85.1 ± 4.1 | 83.6 ± 0.2 | 50.3 ± 0.6 |
| 50 | 89.2 ± 2.9 | 92.3 ± 0.5 | 92.7 ± 0.7 |

[a] Results are expressed as mean ± SD of 4 – 6 determinations.

## Discussion

Routine PDT consists of application of a photosensitizer and subsequent irradiation of the tumor with light that is absorbed by the photosensitizer [2,3]. It has been reported that only certain types of cells become photosensitized when exposed to ALA and others do not. The *in vivo* and *in vitro* studies have reported that PDT could be an effective, non-invasive and safe treatment of superficial bladder cancer [4]. All three human transitional bladder tumor cell lines used in this study demonstrated high photosensitivity. The photocytotoxicity revealed a strong dependence on the ALA concentrations as well as on light intensity. The antitumor effects of PDT using ALA in the bladder cancer cell lines SW780, 647V and T24 were independent of the tumor cells differentation. The results show that ALA-induced photosensitization has a high potential for photodynamic therapy of bladder carcinoma.

## References

1. Pashos C.L., Botteman M.F., Laskin B.L., Redaelli A.: Bladder cancer: epidemiology, diagnosis and management. *Cancer Pract.* **10**: 311-322, **2002**.
2. Almeida R.D., Manadas B.J., Carvalho A.P., Duarte C.B.: Intracellular signaling mechanisms in photodynamic therapy. *Biochim. Biophys. Acta.* **1704**: 59-86, **2004**.
3. Manivasager V., Heng P., Hao J., Zheng W., Soo K., Olivo M.: A study of 5-aminolevulinic acid and its metylester used *in vitro* and *in vivo* systems of human bladder cancer. *Int. J. Oncol.* **22**: 313-328, **2003**.
4. Jichlinski P., Lesinger H.: Photodynamic therapy in superficial bladder cancer: past, present and future. *Urol. Res.* **29**: 396-405, **2001**.
5. Cell proliferation kit I (MTT). *Instruction manual.* Version 3. Roche Applied Science, Germany, **2003**.
6. Cytotoxicity detection kit (LDH). *Instruction manual.* Version 5. Roche Applied Science, Germany, **2004**.