Global DNA methylation status of colorectal cancer cells exposed to photodynamic therapy

L Vorster, N Houreld and H Abrahamse[1]
Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, P.O. Box 17011, Doornfontein, Johannesburg, 2028, South Africa Tel: +27 11 559-6406 Fax +27 11 559-6884,
e-mail: habrahamse@uj.ac.za

Abstract. *Aim:* Photodynamic therapy (PDT) is the use of low intensity laser irradiation (LILI) in conjunction with a photosensitiser (PS), in this instance Zinc (II) Phthalocyanine (ZnPc), to treat cancer cells. A single wavelength is used to activate the PS, which in turn causes changes in cellular functions. DNA methhylation is an epigenetic regulator. The methylation status of a gene determines if it is expressed or silenced. This study aims to determine if DNA methylation has an effect on PDT. *Method:* Cancer cells, demethylated with aza-5-dc and normal, were exposed to PDT with different incubation times. Cell viability, proliferation and morphology were measured. *Results:* Cells exposed to PDT and aza-5-dc showed a statistically significant decrease in viability when compared to control cells. A significant decrease was also shown when cells, demethylated with a high concentration of aza-5-dc were compared to cells exposed to PDT. *Conclusion:* The DNA methylation status of cells does have an effect on PDT.

1. Introduction

DNA methylation is a chemical change to the DNA, without changing the sequence. Hypermethylation normally leads to the silencing of genes and hypomethylation leads to over expression [1-3]. DNA methylation is not only an epigenetic regulator, but also contributes to the tertiary structure of the DNA double helix [4,5]. DNA methylation occurs when a methyl group (CH_3) is covalently added to the 5' position of a cytosine that is part of a CpG di-nucleotide. This methylation is normally found in cytosine and guanine rich regions in the genome called CpG islands [6,7]. Recent studies have shown that the use of a demethylating agent with chemotherapy on a therapy resistant brain tumour increased the chemosensitivity [5]. Low intensity laser irradiation (LILI), also known as photobiostimulation, has been used for various medical treatments. This involves the use of a single wavelength that emits no sound, heat or vibration to stimulate cells. Photodynamic therapy (PDT) is the use of LILI in conjunction with a photosensitiser (PS). The PS is activated by the LILI and it generates reactive oxygen species (ROS) that destroys the cells. In recent years a lot of research has gone into the use of metallophthalocyanine (MPc) as PSs [8].

[1] To whom any correspondence should be addressed.

2. Methodology

Colorectal ademocarcinoma cells (CaCo2, ATCC HTB-37) were cultured in minimum essential media eagle (Sigma M2279) until confluent. After confluence was achieved, cells were treated with aza-5-dc (Sigma A3656) to demethylate the cells at 0.1 µM and 3 µM. Demethylated cells were then seeded into 3.3 cm diameter culture plates and incubated for either 3 or 24 h with 20 µM Zinc phthalocyanine (ZnPc). When seeded into small plates for irradiation two groups were made with different incubation times of the PS, 3 h and 24 h. Cells were then irradiated with 5 J/cm^2 at 680 nm (43.0 mW; 5.03 mW/cm2; 16.5 min; 990 s). Viability was determined using Trypan Blue staining in conjunction with the Countess Automated Cell Counter (Invitrogen C10227). Cell proliferation was measured using the AlamarBlue stain and the Victor^3 multi-label plate reader (PerkinElmer). Cell morphology was viewed using inverted light microscopy.

3. Results and Discussion

3.1 Morphology

Morphological changes were observed as shown in figure 1. The group exposed to PDT where, in the 3h group, the cells became round and started to detach from the plate surface and the 24 h PDT group showed signs of severe structural damage. The control groups (Control, PS, aza-0.1 and aza-3) showed almost no morphological changes. An interesting observation is the vacuoles in the cells. Cells exposed to a high concentration of aza-5-dc (group aza-3 in figure 1) showed a decrease in these vacuoles.

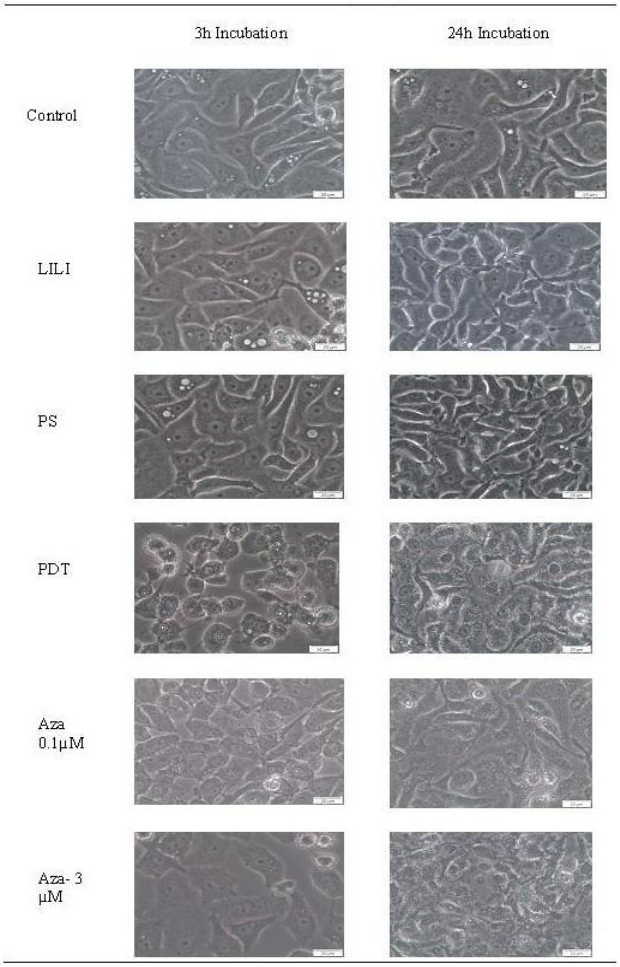

Figure 1: Photographic indication of the morphological changes in the different groups. Groups that served a control are LILI: cells exposed to laser irradiation; PS: cells treated with the photosensitiser. PDT: cells that were treated with PDT. Aza- 0.1: cells treated with a low concentration (0.1µM) aza-5-dc. Aza-3: cells treated with a high concentration (3 µM) aza-5-dc. Aza- 0.1 PDT and Aza-3 PDT are the groups treated with aza-5-dc and PDT.

3.2 Viability and proliferation

Cells exposed to PDT showed a significant statistical difference in decrease of viability when compared to the control at both 3 and 24 h ($P < 0.05$ and $P < 0.001$ respectively) as shown in figure 2a and 2b. In the 24h group, demethylated cells showed a significant difference when compared to the control, but less than the PDT group, as for the 3h group, it is the exact opposite. However when comparing the PDT group to the demethylated groups no significant difference was found, which correlates with the results found by Patties *et. al.*[5]. The cells exposed to a high concentration of aza-5-dc and PDT showed a significant difference when compare to the demethylated cells not exposed to PDT, but it was still less than the PDT group. The viability results show that PDT significantly decreases the viability of cells exposed to it when the PS is incubated for 24h. DNA methylation did not affect PDT when incubated for 24 h. This can be due to the fact that the DNA repair mechanisms had time to work before PDT was administered. Another factor that can play a role in the effectiveness of the aza-5-dc and PDT is the stage of the cell cycle the cells were in [9].

No significant statistical difference was found with the AlamarBlue proliferation assay (results not shown).

(a) (b)

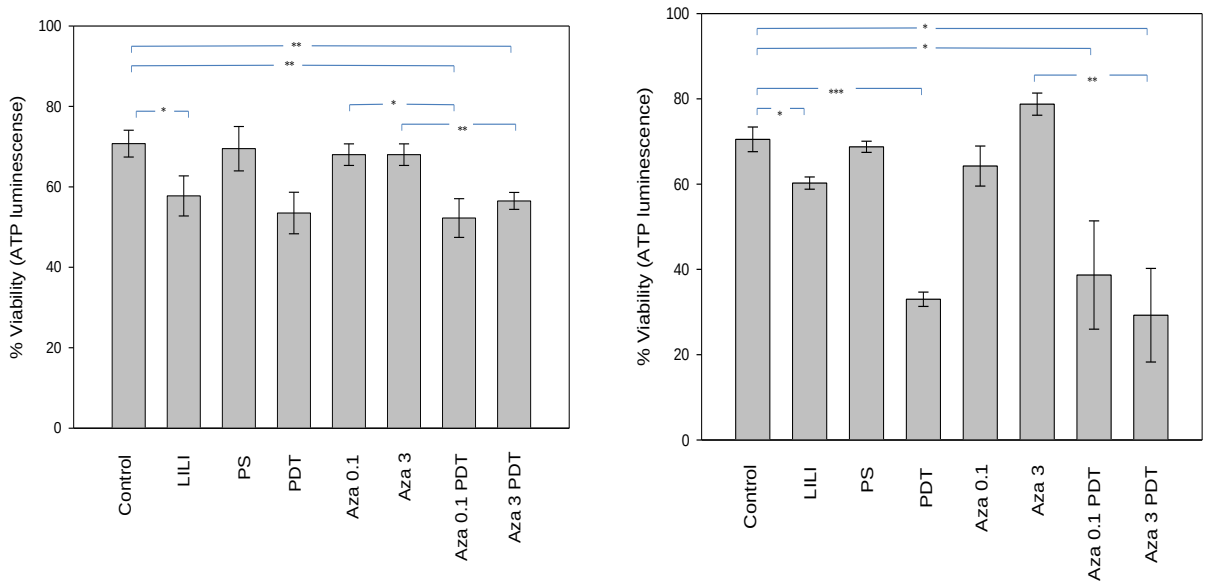

Figure 2(a) and (b): CaCo2 cells were treated with different concentrations of demethylser, aza-5-dc (Aza-0.1 and Aza-3), and incubated with ZnPc for 3 (a) or 24 (b) h. Cells were then irradiated at 660 nm with 5 J/cm2 (PDT). Cells which were not treated (control), irradiated only (LILI), or incubated with ZnPc but not irradiated (PS) served as controls. Statistical significances are shown as *P<0.05, **P<0.01 and P<0.001 (n = 4).

4. Conclusion

From these results, it can be concluded that DNA methylation status does affect PDT, but it is dependent on the incubation time of the PS. If cells are left to incubate for too long, demethylation has no effect on PDT and this is possibly due to activation of cellular DNA repair mechanisms. These results have also shown that CaCo2 cells are sensitive to treatment with 20 μM ZnPc and irradiated to a wavelength of 680 nm and a fluence of 5 J/cm2.

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