

The efficiency of Hypericin used in photodynamic therapy to induce the cell death of human breast cancer cells (MCF-7)

NE Kiro and H Abrahamse

Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, P.O. Box 17011, Doornfontein, South Africa, 2028, Tel: 27(0)115596406

Email: habrahamse@uj.ac.za

Abstract. *Background:* Breast cancer is a life threatening heterogeneous disease, which is currently the second most common invasive cancer that affects women worldwide, after lung cancer. In addition to cancer recurrence, another challenge encountered during cancer therapy is the toxic effects cancer drugs have on healthy cells. Photodynamic therapy (PDT) offers targeted treatment of cancer cells using low intensity light (600-900 nm) in synergy with a photosensitizer (PS). A PS is itself, a nontoxic drug and only becomes toxic to cells in the presence of light, at a specific wavelength, by inducing an overproduction of reactive oxygen species (ROS), which destroys cancer cells. The efficiency of the PS Hypericin (HYP) to induce cancer cell death after its activation was investigated in this study. *Methods:* A Commercially purchased breast cancer cell line (MCF-7) was treated with four different doses of HYP: 0.2 μM , 2 μM , 4 μM and 6 μM , and irradiated with three fluencies: 5, 10 and 15 J/cm² using a 594 nm diode laser. The effect was determined by assessing viability (trypan blue staining), proliferation (Adenosine triphosphate, ATP, luminescence assay), toxicity (Lactate Dehydrogenase, LDH) and cell death pathways (Caspase 3/7) of the breast cancer cells. *Results:* A Change in cellular morphology was seen in the PDT treated cells using fluencies of 10 and 15 J/cm². A decrease in viability and proliferation, and an increase in cytotoxicity and caspase activity were also observed. *Conclusion:* HYP was identified as an efficient PS as it, together with LILI, was able to induce photo damage in MCF-7 cells. The most effective treatment combination was observed using 2, 4 and 6 μM of HYP and a fluence of 15 J/cm².

1. Introduction

Cell division is a meticulously controlled process of the human organism. Tight regulation of this process prevents the growth and proliferation of faulty or mutated cells, thus preventing tumor formation. Cancer is a generic term given to a category of diseases characterized by the dysregulation of several regulatory genes leading to the uncontrolled growth and proliferation of the mutated cells in any part of the body. The disordered gene expression can either be caused by endogenous or exogenous factors inducing the genetic or epigenetic alterations of the concerned genes or closely linked DNA sequences involved in their regulation [1]. Cancerous cells are initially healthy cells that have undergone hyperplasia (increase in the proliferation rate with no structural changes) or atypical hyperplasia (alteration in shape, size and organization) [13]. They can either stay in their tissue of origin (benign or localized cancer) or spread to other part of the body through the vascular and lymphatic system (metastatic or malignant cancer) [2]. Benign cancers are more responsive to conventional treatment including surgery, radio and chemotherapy contrary to malignant cancers which usually show resistance to those approaches and have more detrimental effects [3]. Breast cancer is the most common invasive cancer mostly affecting women than men and the second leading

cause of women death from cancer worldwide [4]. Cancer classification is a crucial step in the choice of the appropriate therapeutic approach. Clinically, breast cancers are classified into four main subtypes; lumina A, lumina B, human epidermal growth factor receptor 2 (HER2 or ErbB2) and triple negative/basal-like breast cancers (TNBC/ BLBC) [5]. Factors such as, the expression of certain protein receptors and the immunohistochemical characteristic of breast cancer cells are to be taking into consideration when assigning the breast cancer into one of the above categories which all have different prognosis [6-7]. During conventional therapeutic approaches of breast cancer (radiation, chemo and hormonal and targeted therapy) healthy cells are also killed leading to the subsequent side effects [8]. The selective cancer cells eradication that offers PDT, a novel light dependent therapeutic modality, allows keeping healthy cells surrounding the target tumor cells unharmed after treatment [9]. Hence minimizing the side effects. PDT uses in synergy low intensity light, a photosensitizer (PS) and oxygen to induce cytotoxicity in targeted cancer cells [10]. The initially nontoxic PS acquires its toxicity upon illumination with a light of the appropriate wavelength matching its absorption spectrum [10]. The ROS overload caused by the phototoxic PS is believed to be the key priming event in the induction of the apoptotic cell death mechanism pathway in PDT treated cells [11]. The efficiency of HYP, a naturally occurring selective anti-tumor agent extract from *Hypericum perforatum*, used as PS in PDT has been established in previous in vitro and in vivo cancer treatment related investigations [12]. In the present study, the effectiveness of four different concentrations of HYP used in synergy with laser light was investigated. Afterwards, the HYP and light dosage combination capable of inducing the higher percentage of breast cancer cells death in vitro was assessed.

2. Methods

A breast cancer cell line (MCF-7) was used and cells were seeded into 3.4 cm² diameter culture dishes, followed by a 4 hour incubation to allow them to attach. Cell cultures were separated into 4 groups; untreated control cells, drug only control, laser only control and the experimental groups (PDT treated cells). Four different concentrations of HYP (0.2, 2, 4 and 6 μ M) and four doses of light (5, 10 and 15 J/cm²) were used. The drug administration and laser irradiation were performed in the dark. A 594 nm diode laser was used and its parameters are presented in **Table 1** was used. All samples were incubated for 24 hours after which cell responses were assessed. *Cell viability*: Percentage viability was determined using the trypan blue staining technique. *Cell proliferation*: The proliferation rate was evaluated by recording the luminescence signal using the adenosine triphosphate (ATP) luminescence assay. *Cytotoxicity*: Membrane integrity was determined by measuring the lactate dehydrogenase (LDH) at 490 nm. *Statistical analysis*: Significant differences were considered at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). All chemicals used were of research grade.

Table 1 Parameters of the laser used in this study

Parameters	Diode laser
Manufacturer	Optoelectronics Tech. Co., LTD, China
Wavelength	532 nm
Wave emission	Continuous
Spot size	9.1 cm ²
Output power	642 mW
Power density	75.06 mW/cm ²
Fluencies	0 J/cm ² 5 J/cm ² 10 J/cm ² 15 J/cm ²
Irradiation times	0 min, 0 sec 6 min, 24 secs 12 min, 49 secs 19 min, 13 secs

3. Results and discussion

Morphological changes and detachment from the culture dishes were observed under the microscope in the PDT treated. Cells treated with laser or HYP only did not show any morphological change. This indicated that the drug successfully acquired toxicity upon laser illumination, interacted with molecular oxygen within cancer cells and induced their subsequent damage.

The ratio of viable to dead cells was obtained by performing the Trypan blue exclusion assay as shown in **Figure 2**. Lower percentage viability was observed in PDT treated cells as compared to the untreated ones. The proliferation rate was assessed using the ATP Luminescence assay. As presented in **Figure 3**, a significant decrease in cell proliferation upon laser irradiation using 5, 10 and 15 J/cm² was observed with 2, 4 and 6 μ M. A drug concentration of 0.2 μ M induced a decrease in cell proliferation only after irradiation with 15 J/cm². A slight decrease in cell proliferation was also noted after treating cells using 2, 4 and 6 μ M of HYP in the absence of laser. Further studies will be done to assess the HYP dark toxicity. The membrane integrity was examined by means of LDH release into the culture media. Cells treated with 2, 4 and 6 μ M of HYP released a higher quantity of LDH post laser irradiation as shown in **Figure 4**. The decrease in cell viability and ATP production coupled with the membrane degradation observed in PDT treated cells support previous research reports of the photo-damaging effect of HYP on cancer cells [12]. The slight increase in cell proliferation, post laser irradiation, in the absence of HYP confirms the bio-modulative effect of laser at a cellular level [10].

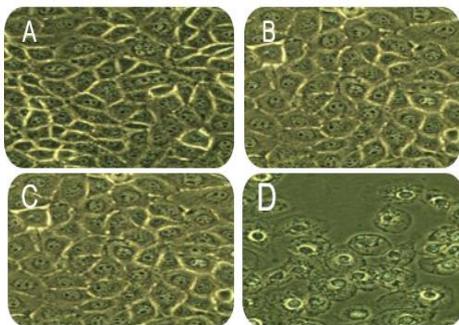


Figure 1. The morphological characteristics of the untreated control cells (A), cells treated with laser only (B), cells treated with drug only in the absence of laser (C) and PDT treated cells (D). A change in shape was observed in PDT treated cells. This indicated their damage upon receiving a combination of drug and laser treatment.

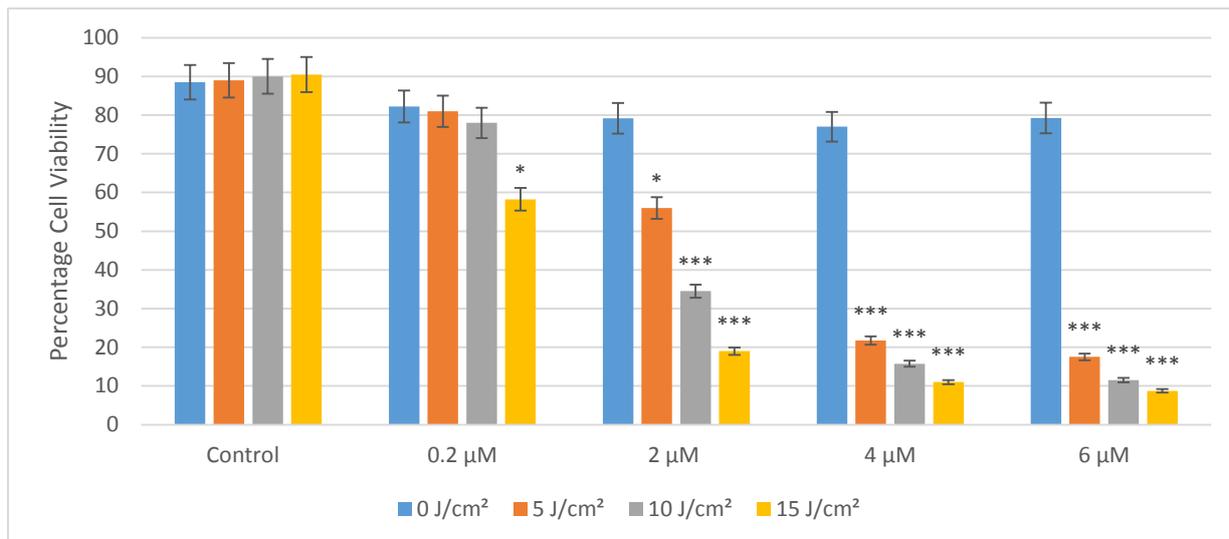


Figure 2. Cell viability study using the Trypan blue exclusion test. Upon treatment with different concentration of HYP, the percentage viability decreased in the light dependent manner when compared to the reference untreated control. Statistical differences were indicated as *, ** and *** compared to the reference control group.

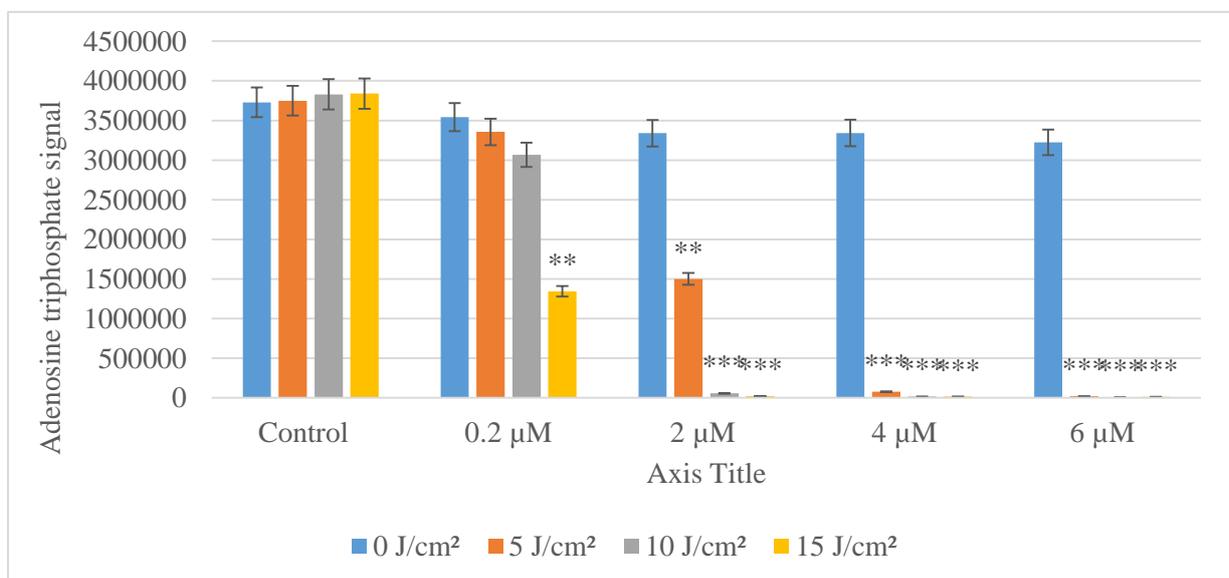


Figure 3. Cell proliferation study using the adenosine triphosphate (ATP) luminescence assay. The rate of proliferation significantly decreased using drug concentration from 2 μM with all three doses of light and 0.2 μM with 15 J/cm² compared to the untreated control. Statistical differences were indicated as *, ** and *** compared to the reference control group.

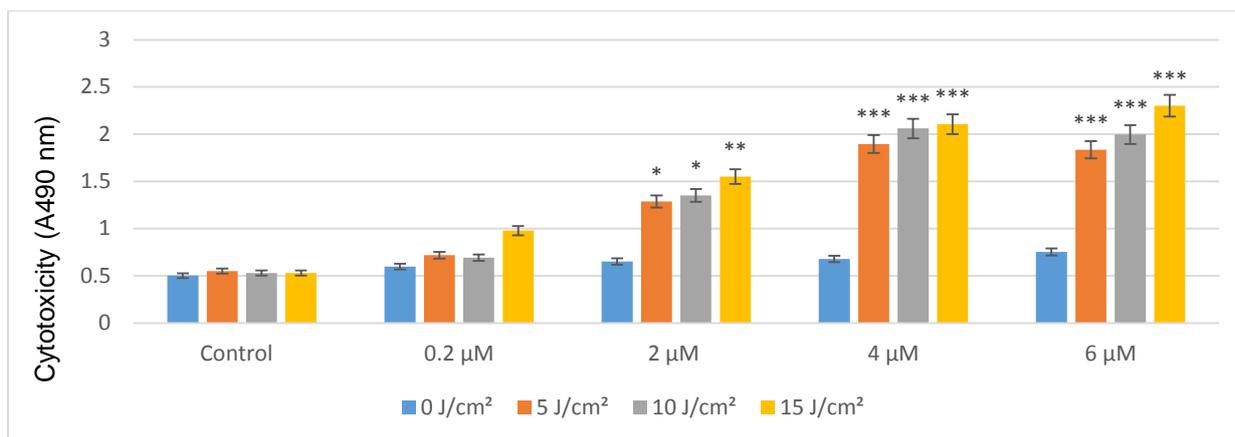


Figure 4. Membrane integrity study using the lactate dehydrogenase (LDH) cytotoxicity assay. Obvious increase in cyto-damage was observed post laser illumination using drug concentration from 2 μM when compared to the untreated control. Statistical differences were indicated as *, ** and *** compared to the reference control group.

4. Conclusion

This in vitro study has shown the highest photodynamic effect of HYP with 2, 4 and 6 μM using fluencies of 5, 10 and 15 J/cm^2 . This implies that HYP is an efficient PS, if used in a dose-dependent manner. Therefore, since PDT aims at using the lowest concentrations of PS, 2 μM could be suggested for possible clinical trials.

5. Acknowledgments

This work was conducted at the Laser Research Centre of the University of Johannesburg and supported by all the African Laser Centre, National Research Foundation of South Africa, South African Research Chairs Initiative, DST/NRF SARChI Research Chairs and the University of Johannesburg. The National Laser Centre of South Africa is acknowledged for the supply and maintenance of lasers.

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