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Photodynamic Therapy to Eradicate Tumor Cells

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1. Introduction

The cell cycle is a collection of highly ordered processes involving numerous regulatory proteins that guide the cell through a specific sequence of events culminating in the duplication of the cell (Elledge, 1996). In general, the cell cycle can be altered to the advantage of many factors. Three basic cell cycle defects are mediated by misregulations of cyclin-dependent kinases (CDKs), unscheduled proliferation, genomic instability (GIN) and chromosomal instability (CIN). In the first case, mutations result in constitutive mitogenic signaling and defective responses to anti-mitogenic signals. The second, GIN, leads to additional mutations (Kastan & Bartek, 2004), and CIN is responsible for numerical changes in chromosomes (Lee et al., 1999). Moreover, data suggest that the mutations leading to tumorigenesis are even more numerous and heterogeneous than previously thought (Hudson et al., 2010). This accumulation of genetic mutations can arise by nucleotide substitutions, small insertions and deletions, chromosomal rearrangements and copy number changes that can affect protein-coding or regulatory components of genes. Cancer genomes usually acquire somatic epigenetic “marks” compared to non-neoplastic tissues from same organ (Hudson et al., 2010). In this context, a neoplasm (Greek, *neo* = new + *plasis* = growth) can be defined as an abnormal mass of tissue whose growth exceeds and is uncoordinated with that of the normal tissue, and persists in the same excessive manner after cessation of the stimuli that initiated the change.

Head and neck cancer is considered a worldwide problem due to its raising in developing countries (Lim et al., 2011). Approximately 90% of this type of cancer consists of oral squamous cell carcinoma (OSCC), which arises from the oral mucosal lining (Neville & Day, 2002). The risk factors related to OSCC includes tobacco and alcohol abuse, solar exposure, human papillomavirus, immunosuppression conditions, iron deficiency anemia in combination with dysphagia and esophageal webs, and tumor genesis that can occur as a result of genetic predisposition or epigenetic pathway involving DNA and/or histone modification (Neville & Day, 2002; Schweitzer & Somers, 2010). Although there have been

many advances in the conventional treatment, the survival rate for patients with late stage of OSCC is the lowest of the major cancers, remaining at 50% over the last two decades (Neville & Day, 2002; Funk et al., 2002). The standard treatment for early OSCC includes surgery, radiation, chemotherapy or a combination of these procedures. However, the side effects of these treatments are severe and can result in structural defects leading to dysphagia, and also hyperpigmentation, scars and xerostomia (Hooper et al., 2004; Schweitzer & Somers, 2010). Therefore, alternative treatments have been proposed in order to reduce the toxicity and side effects from the conventional therapies.

Less invasive surgical modalities including the Photodynamic Therapy (PDT) are examples of new treatments that comprise the era of conservative surgery (Karakullukcu et al., 2011). In this context, the purposes of this chapter are: 1. discuss about PDT as a relatively new therapy for treating head and neck neoplasms including its advantages and disadvantages when compared to conventional treatments; 2. describe the pathophysiology of cancer cells that allows the photosensitizer to accumulate on these cells rather than normal tissue and mechanisms of cell/tumor death; 3. review the *in vivo* findings available in the literature; and 4. present the anti-tumor effect observed *in vitro* when HeLa cells were exposed to Curcumin, a natural photosensitizer.

2. Photodynamic therapy

PDT is a relative recent therapy, and excellent results have been reported after its application for the treatment of oral cancer and premalignant lesions (Allison et al., 2005; Yu et al., 2008), as well as bacterial and fungal infections (Teichert et al., 2002; Williams et al., 2006; Donnelly et al., 2008). The simplicity of PDT mechanism stimulated the interest for this therapy, which is characterized by the association of a photosensitizing agent (PS) and visible light with a wavelength compatible with the photosensitizer's absorption spectrum (Konopka & Goslinski, 2007; Buytaert et al., 2007). Photon absorption by the PS leads it to a triple state of excitation that may interact with the available oxygen, in two different ways. The reaction type 1 involves electron/hydrogen transfer directly from the PS or electron/hydrogen removal from a substrate molecule to form free radicals such as superoxide, hydroxyl radicals, and hydrogen peroxide. The reaction type 2 involves the production of the electronically excited and highly reactive state of oxygen known as singlet oxygen (Konopka & Goslinski, 2007). Both reactions can occur at the same time and the PS' characteristics and the substrate molecules are important components to define the ratio of each reaction. All these products originated from PDT may result in a cascade of oxidative events that cause direct cell death, destruction of tumor vascularization and activation of the host's immune response (Buytaert et al., 2007).

The success of PDT depends on several parameters such as the type and concentration of the PS, its localization during the irradiation, the pre-incubation period with the drug, type of light sources, light fluence and density, type of tumor and its level of oxygenation (Dolmas et al., 2003). Regarding the PS, the most commonly used in PDT for cancer treatment includes porphyrins, phthalocyanines, the 5-aminolevulinic acid and chlorine. These PSs have been preferentially selected due to their approval for clinical use. In the present, PDT has been approved for use in clinical treatment in the USA, EU, Canada, Russia and Japan (Bredell et al., 2010). The Food and Drug Administration (FDA) has approved the treatment of Barrett's esophagus, obstructing esophageal and tracheobronchial carcinomas with

Photofrin, a hematoporphyrin derivate. Moreover, it has also approved the treatment for actinic keratosis with Levulan (aminolevulinic acid) and for macular degeneration with Verteporfin (benzoporphyrin derivate monoacide). In the EU, the same conditions have been approved adding the treatment for early head and neck cancer and palliative treatment for this type of cancer with Foscan, a tetrahydroxy-phenyl chloride (mTHPC) (Biel, 2007). This diversity of photosensitizers leads to differences in the PDT protocol, including differences in the dose and pre-incubation period with the photosensitive drug.

The pre-incubation period is extremely important in the photosensitizer toxicity and its intracellular localization. Hsieh et al. (2003) observed that when Photofrin remained in contact with tumor cells for one hour, the PS was retained in the plasma membrane; however, when this period was increased to 24 hours, the PS penetrated into the cells and caused cell damage even in the absence of light (Hsieh et al., 2003). Triesscheijn et al. (2004) found that a lower light dose was sufficient to reduce cell viability when a higher pre-incubation time was used for several cell types. Moreover, the PS localization defines the primary site of photodamage and also the type of cell death occurred after PDT. Generally, the PSs that accumulate in mitochondria or endoplasmic reticulum cause apoptosis, while those that accumulate in the plasma membrane or lysosomes predispose cells to necrosis (Buytaert et al., 2007). Also, PDT can induce autophagy in attempt to repair the photodamage and turn into a cell death signal if the initial response fails (Kessel & Oleinick, 2009). Usually the PSs do not accumulate in the nucleus, which results in a low mutagenic and genotoxic potential of PDT. A study evaluating DNA damage after PDT observed that the treatment with methylene blue associated with halogen light irradiation against a keratinocytes culture did not cause any DNA damage after 4 hours, even with a 90% percentage of cellular lysis (Zeina et al., 2003).

Light is an essential component of PDT reaction and it is able to interact and modify some cellular responses. According to Brancalion & Moseley (2002), the choice of an adequate light source can be dictated by the location of the tumour, the light dose required and the type of the PS used. PDT has been traditionally performed using lasers (Light Amplification Stimulated Emission). Examples of this category of light are the argon, argon-pumped dye, metal vapour-pumped dye, solid state, and diode lasers. However, the use of these large and complex equipments can be limited due to the expensive technologies and maintenance required. Because of such disadvantages, lasers are not the only light sources that have been investigated for photodynamic purposes. Studies have reported the use of halogen light and LED (Light Emitting Diode). The LED is a category of light source that emits radiation in a wider range of the spectrum, but with a dominant wavelength. Moreover, the device has a lower cost and simpler technology compared to laser.

The main advantage of PDT is the limited tissue damage restricted to the illuminated and photosensitized area without long-term systemic side effects (Cooper et al., 2007; Karakullukcu et al., 2011). It also does not cause damage to normal structures like nerves, collagen fibers and large blood vessels, preserving the supporting components (Bredell et al., 2010). This means that when cancer cells are eliminated via apoptosis/necrosis the extracellular matrix remains forming a scaffold for the surrounding mucosal tissue to advance over, resulting in minimal scar formation. Moreover, this therapy can be repeated as often as needed without accumulative destructive effects and there is no interaction between current chemo- and radiotherapy protocols and PDT. The most common

disadvantages are the photosensitivity that can lead to burn wounds, pain that can vary from mild to severe resolving within 2-3 weeks, and possible poor initial selectivity between tumor and normal tissue (Biel, 2007; Bredell et al., 2010). Although PDT presents some local side effects, it does not involve systemic or serious adverse events. Despite these advantages compared to the conventional treatments, the lack of clinical knowledge and especially the lack of established treatment protocols lead to the limited application of this treatment in the head and neck cancer field.

3. Characteristics of tumor cells and mechanisms of tumor destruction by PDT

The effectiveness of PDT treatment depends on the ability of the PS to selectively localize in tumor cells as opposed to normal cells. The selectivity of PDT to tumor cell is a result of some important characteristics of these cells such as greater proliferative rates, leaky vasculature, poor lymphatic drainage, high expression of low-density lipoprotein (LDL) receptors on tumor cells, to which the PS can bind, and low pH facilitating cellular uptake of the PS.

The antitumor effects of PDT against tumor cells include the direct cytotoxic effect, damage to the tumor vasculature, and induction of inflammatory reaction (systemic immunity) (Agostinis et al., 2011). Three PDT death pathways are described: apoptosis, autophagy and necrosis. The apoptosis mechanisms remain obscure and complex. Kessel & Luo (1998) described that apoptosis occurred when PS was accumulated in the mitochondria, but not when PS was located in the cytoplasmic. Furre et al. (2006) showed that PDT can induce apoptosis through both cytochrome c-mediated caspase-dependent and AIF-induced caspase-dependent pathway. In addition, Baglo et al. (2011) described that PDT triggers a complex cellular response involving several biological pathways, but apparently involve both caspase dependent and independence apoptotic pathways. Specifically in head and neck cancer, PDT seems to active caspase-8 and caspase-9 pathway and their upstream NF- κ B-JNK pathways (Chen et al., 2011). In summary, lysosomal membrane rupture and leakage of cathepsins involve mitochondria outer membrane permeabilization (MOMP) controlled by Bcl-2 family members. Autophagy is a complex cellular process involving dynamic membrane rearrangements and degradation of cell components through the lysosomal activity related to energy homeostasis, organelle turnover, and cancer death (Stromhaug & Klionsky, 2001). Buytaert et al. (2007) identified autophagy as a mode of cell death after PDT. While PDT treated murine embryonic fibroblasts WT (wild-type) and DKO-mtBAX (DKO reexpressing mtBAX) cells exhibited cellular shrinkage and membrane blebs characteristic of apoptotic cell death, the DKO (double-knockout, BAX and BAK) cells and DKO-SERCA (DKO overexpressing SERCA2) cells readily showed extensive cytosolic vacuolization which was remarkably prevented by class III phosphatidylinositol 3-kinase (PI3K), necessary for the sequestration of cytoplasmic material on autophagy process (Buytaert et al., 2007). The mechanisms and roles of autophagy following PDT could also differ markedly in different cell types, depending on a variety of cell characteristics, including their propensity to undergo apoptosis and in response to agents that produce different types or locations of damage and its lead to more resistant (organelle turnover) or death of cancer cells (Kessel & Oleinick, 2009). For necrosis, the PS is excited by light and then reacts with oxygen to generate reactive oxygen species (ROS) that ultimately lead to

cell death. ROS can lead damage to proteins, nuclei acids and lipids, proteins change functions with attack to disulfide bonds and thiol groups. Another effect of ROS could be the modification of Ca^{2+} channels (Vanlangenakker et al., 2008). The increase of permeability of mitochondrial membrane is probably a dynamic process that can activate apoptosis or succumb to necrosis (Gramaglia et al., 2004). Mitochondria controls cytoplasmic concentration of Ca^{2+} by tuning the frequency of cytosolic Ca^{2+} waves, and this mechanism is potentially lethal and would rapidly activate a variety of enzymes in an uncontrolled fashion (Rasola & Bernardi, 2011). The fact that cancer cells can die through different mechanisms is a relevant clue in the choice and design of anticancer PDT (Panzarini et al., 2011).

The antivasular effects of PDT. Star et al. (1986) described direct evidence of endothelial damage from the histological sections of blood vessels showing destruction of the vessel wall. The effects observed were blanching (ischemia) of tumor, the circulation gradually slows down, leading to complete stasis (hour to day after irradiation), vasodilatation and hemorrhage (dose related) (Star et al., 1986). The directly cytotoxicity of endothelial cells were described with sublethal doses of PDT. Gomer et al. (1988) showed that bovine endothelial cells were significantly more sensitive than smooth muscle cells or fibroblast from the same species and conclude that endothelial cell photosensitivity may play a role in the vascular damage observed following PDT.

The inflammatory and immune response: The PDT oxidative stress and associated cell damage explain the strong acute inflammatory reaction. Moreover, the acute inflammation is characterized by increased expression of several pro-inflammatory cytokines including TNF- α , IL-1 β , and IL-6, adhesion molecules E-selectin and ICAM-1, and rapid accumulation of leukocytes into the treated tumor bed (Brackett & Gollnick, 2011). It also involves the maturation and activation of dendritic cells, and ability to stimulate T cell activation (Brackett & Gollnick, 2011). Pre-clinical and clinical studies have demonstrated that PDT eliminates tumors by direct tumor cell death and indirectly by augmenting anti-tumor immunity. An *in vitro* study showed that PDT caused the suppression of several tumour-promoting factors in head and neck cancer such as MMP-2, MMP-9, uPA and VEGF. The study do not confirm whether PDT reduces the invasive potential of malignant head and neck cells, but it presents the suppression of factors responsible for tumor invasion which may be of therapeutic value (Sharwani et al., 2006).

4. *In vivo* findings for head and neck cancer treatment with PDT

Several clinical studies have evaluated the potential of PDT for treating head and neck cancer using approved photosensitizers (Cooper et al., 2007; Biel, 2007; Schweitzer & Somers, 2010; Karakullukcu et al., 2011). In a study reported by Biel (2007), 276 patients with early head and neck squamous carcinoma were treated with Photofrin, a first generation PS. After 48 hours, the light exposition was performed with laser using different light fluencies and densities depending on the localization of the tumor (oral cavity, larynx) under local anesthesia. The cure rates with a single treatment for early laryngeal and oral cancers were 91% and 94%, respectively. A study using the same PS showed that PDT was able to provide local control in 80% of cases with diffuse aggressive mucosal carcinoma *in situ* of the oral cavity/oropharynx, especially in patients in which conventional treatment failed (Schweitzer & Somers, 2010).

The second generation of PSs was improved in order to give greater tumor selectivity and allow deeper light penetration into the tissue as a result of its longer excitation wavelength (Allison et al., 2004). Foscan, a chlorin, was the only second-generation PS studied in clinical trials (Biel, 2007). A recent study analyzed 170 patients with 226 lesions that included primary (95) and non-primary (131) oral cavity and oropharynx neoplasms. It was observed an overall response rate of 90.7% with a complete response rate of 70.8% (Karakullukcu et al., 2011). This study also revealed the difference in response rates between different regions in the oral cavity. For example the tongue reacts significantly better than alveolar process, showing that some sub-sites present better characteristics for PDT as flat surface for a more homogenous light deliver. Cooper et al. (2007) evaluated 27 patients with secondary or multiple primary head and neck cancer treated with mTHPC intravenously. After 4 days, the tumor area was irradiated with a 652 nm laser under general anesthesia. After 24 hours from the treatment, necrosis and formation of slough at the tumor area was observed. In 28 of the 42 tumors, a complete remission was obtained which represents a complete response of 67%. The authors observed a great difference between the cure rates for stage I/in situ (85%) when compared to stage II/III (38%). These results emphasize the importance of an early diagnosis and periodic follow-up in order to discover new primary tumors in a curable stage.

The clinical studies using ALA mediated PDT showed that this drug has a limited depth accumulation that results in limited penetration of light reducing the application of this therapy. In the study of Fan et al. (1998), 18 patients that were diagnosed with dysplasia and malignant lesions in the oral cavity were treated with ALA mediated PDT, and only two of six patients with tumor obtained a complete response. Moreover, Sireon et al. (2001) observed that only a partial response was obtained after treating patients diagnosed with larynx carcinomas using ALA-PDT. Therefore, more studies with a greater number of patients using correct including criteria should be done in order to evaluate the potential of ALA-mediated PDT to treat head and neck cancer.

5. Evaluation of the antitumor potential of curcumin-mediated PDT

Recently, medicinal plants have become the focus of intense study regarding the prevention and/or treatment of several chronic diseases. Turmeric, the powdered rhizome of *Curcuma longa* L., has been used to treat a variety of inflammatory conditions and chronic diseases (Ammon & Wahl, 1991). Curcumin (CUR) is a naturally occurring, intensely yellow turmeric pigment that it is in worldwide use as a cooking spice, flavoring agent and colorant (Epstein et al., 2010). An increasing number of investigations have suggested that CUR exhibits potential therapeutic applications such as anti-inflammatory, antioxidant, antimicrobial, antifungal and anticancer properties (Epstein et al., 2010; Martins et al., 2009). Some studies also showed that CUR inhibits chemically induced carcinogenesis in the skin, forestomach, and colon when it is administered during initiation and/or postinitiation stages (Kawamori et al., 1999; Huang et al., 1994). Some studies have also propose that these effects may possibly be enhanced by combination with light (Bruzell et al., 2005; Dujic, et al., 2009), thus attracting researches to explore its use in several areas including photochemistry and photobiology. For this reason, an in vitro investigation was conducted to evaluate the association of CUR with LED light for the photoinactivation of a tumor cell line.

5.1 Photosensitizer and light source for PDT

Natural CUR (Fluka Co.) was obtained from Sigma Aldrich, St. Louis, MO, USA. A stock solution of CUR (200 μ M) was prepared in DMSO and then diluted in saline solution to obtain the concentrations to be tested (keeping the final concentration of DMSO at 10%). The 10% concentration of DMSO was selected since has been shown to have no effects on cells viability (data not shown). A light emitting diode (LED) based device, composed of eight royal blue LEDs (LXHL-PR09, Luxeon® III Emitter, Lumileds Lighting, San Jose, California, USA), was used to excite the CUR. The LED device provided a uniform emission from 440nm to 460nm, with maximum emission at 455nm. The irradiance delivered was of 22mW/cm².

5.2 Hela culture, PDT treatments and analysis of cell morphology by SEM

The immortalized Hela cell line, purchased from Adolfo Lutz Institute (São Paulo, SP, Brazil), was selected to perform the anticancer evaluation because it is considered a classic prototype of a cell with high malignancy. The cell line was cultured in Eagle's minimum essential medium (Adolfo Lutz Institute, São Paulo, SP, Brazil.) supplemented with 10% bovine fetal serum (Gibco, Grand Island, NY, USA), with 100 IU/mL penicillin, 100 μ g/mL streptomycin and 2mmol/L glutamine (Gibco, Grand Island) in an humidified incubator with 5% CO₂ and 95% air at 37°C (Isotemp Fisher Scientific, Pittsburgh, PA, USA). The cells were sub-cultured every 3 days until an adequate number of cells were obtained for the study. After reaching approximately 80% density, the cells were trypsinized, seeded in sterile 24-well plates (30.000 cells/cm²) and incubated for 72 hours.

After incubation, the culture medium was removed and cells were washed with phosphate buffer saline (PBS). Aliquots of 350 μ L of CUR at final concentrations of 5, 10 and 20 μ M were transferred individually to wells in 24-well plates and were incubated in contact with the cells for 20 minutes at dark. After the incubation period, cells were irradiated for 4 minutes, corresponding to 5.28 J/cm² (C+L+). Additional wells containing cells exposed only to CUR (C+L-), or only to LED light (C-L+) were also evaluated. Negative control group was composed of cells not exposed to LED light or CUR (C-L-). Cell viability was evaluated by succinic dehydrogenase (SDH) enzyme, which is a measure of the mitochondrial respiration of the cell. In 10 wells, 900 μ L of Eagle's medium associated with 100 μ L of MTT solution (5mg/mL sterile PBS) (Sigma Chemical Co.) was added to the cells cultured in each well and incubated at 37°C for 4h. After this, the culture medium (Eagle's medium with the MTT solution) was aspirated and replaced by 600 μ L of acidified isopropanol solution (0.04N HCl) to dissolve the blue crystals of formazan present in the cells. Cell metabolism was determined as being proportional to the absorbance measured at 570nm wavelength with the use of an ELISA plate reader (BIO-RAD, model 3550-UV, microplate reader, Hercules, CA, USA).

For Hela cell-line morphology analysis by Scanning Electron Microscopy (SEM), sterile cover glasses 12mm in diameter (Fisher Scientific) were placed on the bottom of the wells of all experimental and control groups immediately before the cell seeding. After the experimental conditions, the culture medium was removed and the viable cells that remained adhered to the glass substrate were fixed in 1mL of buffered 2.5% glutaraldehyde for 24hours and post-fixed with 1% osmium tetroxide for 1 hour. The cells adhered to the

glass substrate were then dehydrated in a series of increasing ethanol concentrations (30, 50, 70, 95 and 100%) and immersed in 1,1,1,3,3,3-hexamethyldisilazane (HMDS; Acros Organics, Springfield, NJ, USA) for 90 minutes and stored in a desiccator for 24 hours. The cover glasses were then mounted on metal stubs, sputter-coated with gold and Hela cells morphology was examined by SEM (JEOL-JMS-T33A Scanning Microscope, Tokyo, Japan).

Each experimental treatment was repeated five times. The results obtained presented heterocedasticity. The Kruskal-Wallis and a post-hoc Dunn tests were used to detect differences in absorbance values (MTT) among investigated groups. Differences were considered statistically significant at $p < 0.05$.

5.3 Results

The box plot (Fig. 1) shows the median, 1st and 3rd quartiles, lowest and highest values obtained under each of the experimental and control conditions. Considering the negative control group (C-L-) as having 100% of Hela cell viability, a significant reduction in cell metabolism of 75.5, 81.6 and 87.3% was observed for curcumin concentrations of 5, 10, and 20 μM when the cells were irradiated by the blue LED.

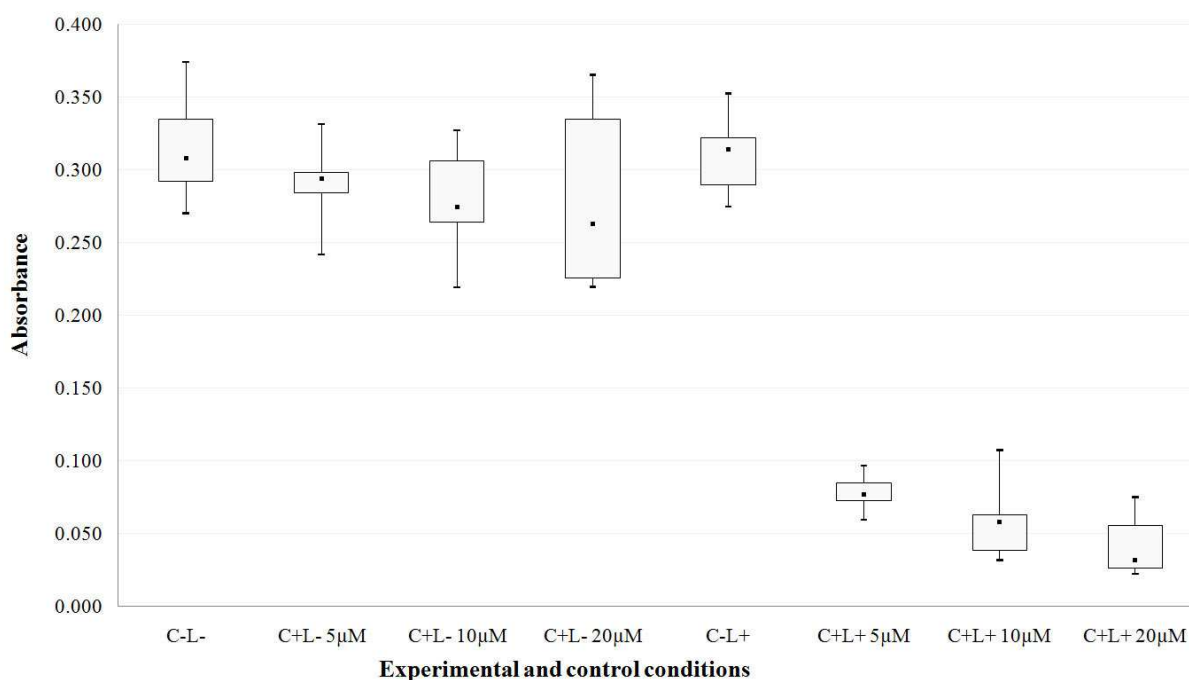


Fig. 1. Summary of absorbance values obtained after experiments with Hela cell culture.

In the C+L+ groups, there was statistically significant difference ($p < 0.05$) between the CUR concentrations of 5 and 20 μM (Table 1). In comparison with the control group (C-L-), the dark toxicity of CUR was observed for the concentrations of 10 and 20 μM (reduction of 11.63 and 11.10% respectively), although no significant differences were detected among the three concentrations. The irradiation control group showed similar metabolism activity to the negative control group, showing that the light dose used in the present study did not alter the cell metabolism.

Experimental and control conditions	Mean Ranks	Post hoc test
C-L-	62.60	a
C+L- 5 μ M	54.50	abc
C+L- 10 μ M	48.70	c
C+L- 20 μ M	51.30	bc
C-L+	60.40	ab
C+L+ 5 μ M	23.15	d
C+L+ 10 μ M	14.55	de
C+L+ 20 μ M	8.80	e

Table 1. Post hoc multiple comparisons of mean ranks for the association of three curcumin concentrations with or without light against Hela cells. Significant differences ($p < 0.05$) among rows are indicated by different small letters.

Figure 2 (a-d) presents a panel of SEM micrographs of the Hela cell-line representative of the control and experimental groups. For the negative control group (no treatment) and the group treated with Curcumin (20 μ M) alone, numerous Hela cells that remained adhered to the glass substrate exhibited wide cytoplasm and numerous fine cytoplasmic processes originated from the cell membrane (Figure 2a,b). In the groups submitted to PDT, there were a smaller number of Hela cells that remained adhered to the coverglass, which can explain the lower cell metabolism observed for the MTT assay. In PDT group using 5 μ M of Curcumin, it can be observed a small number of cells with ill -defined cytoplasmic membrane limits (Figure 2c). However, in PDT group using 20 μ M of Curcumin, it is not observed any cell adhered to the glass substrate. Only rests of cytoplasmic membrane of dead cells were observed, suggesting necrosis death (Figure 2d).

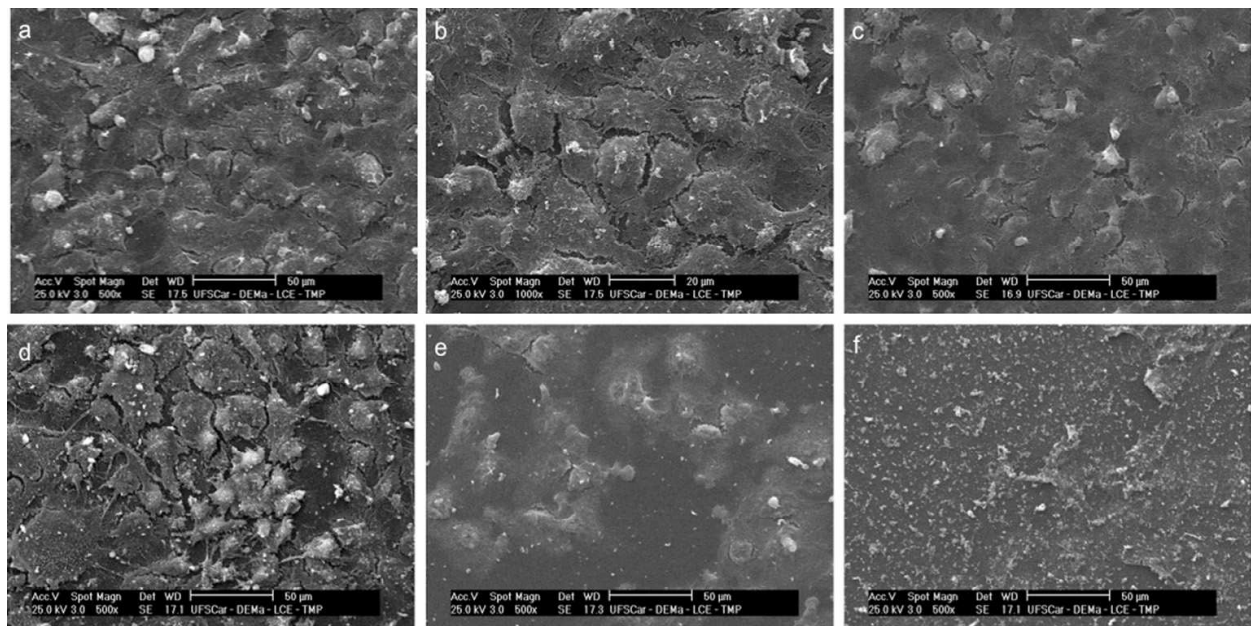


Fig. 2. a- Negative control group of Hela cell line, SEM, 500X; b- Negative control group of Hela cell line, SEM, 1000X; c- Hela cells exposed only to LED irradiation with a light fluence of 5.28 J/cm²; d- Hela cells in contact with Curcumin (20 μ M), SEM, 500X; e- Group PDT with 5 μ M of Curcumin, SEM, 500X; f- Group PDT with 20 μ M of Curcumin, SEM, 500X.

5.4 Discussion

The association of CUR and light achieved a significant reduction in cell metabolism of 87.3%. On the other hand, the effect of CUR without illumination was also evaluated and the results showed a smaller degree of reduction in cell viability, when compared with the PDT results. Consistent with the data of the present study, Koon et al. (2006) reported that the cytotoxicity of CUR was enhanced by the irradiation using blue light with light fluence of 60 kJ/m², although dark cytotoxicity was also observed against a nasopharyngeal carcinoma cell line. The results of Park et al. (2007) also suggest the use of CUR as photosensitizer against skin cancer cells. The mode of action of CUR against tumor cells is likely to be induced by the apoptotic pathway. Curcumin has also been reported to selectively lead to apoptosis in various cancer cells without affecting normal and primary cells (Koon et al., 2006; Park et al., 2007; Weng et al., 2011). As regards the mode of action of photosensitized-CUR, evidences from the literature suggest that cell shrinkage, membrane bleeding and apoptosis can occur in different cell lines (Priyadarsini, 2009). In the present study, PDT-treated cells showed altered morphology, smaller size and smaller number of cells adhered to the cover glass. In addition, there were cell fragments and ill-defined cytoplasmic membrane limits in the cells subjected to PDT. This could be an indicator that the plasma membrane was the main target of the photosensitizer. As a rule, photosensitizer accumulation in the mitochondria or endoplasmic reticulum causes apoptosis, while its accumulation in the plasma membrane or lysosomes predisposes the cells to necrosis (Buytaert et al., 2007). Thus, it can be suggested that curcumin-mediated PDT promoted necrosis of Hela cells, although the type of cell death was not evaluated in the present investigation.

In conclusion, when CUR was irradiated with blue LED light, it proved to be an effective photosensitizing agent for the inactivation of tumor cells. However, some investigations have indicated that the main constraint to *in vivo* application is its low bioavailability, mainly due to its poor absorption in blood and fast metabolism. Thus, future investigations will be conducted to improve the solubility of CUR in an aqueous solution in an attempt to produce a formulation of CUR with adequate bioavailability and clinical efficacy for delivery to cancer cells.

6. Conclusion

According to the information discussed in this chapter, there is scientific evidence to support the efficacy of PDT in treating head and neck tumors. Several protocols of this treatment have been described and discussed, and the results from these studies have shown that PDT can be the option for those patients that have failed prior conventional treatments and an alternative to control multiple primary tumors without the morbidity of surgery or chemo/radiotherapy. PDT has the potential to be an important treatment modality for head and neck cancer due to its minimal side effects and reasonable local control. Therefore, more clinical studies are needed to establish treatment protocols with different photosensitizers as natural compounds in order to increase the application of this therapy in the community settings.

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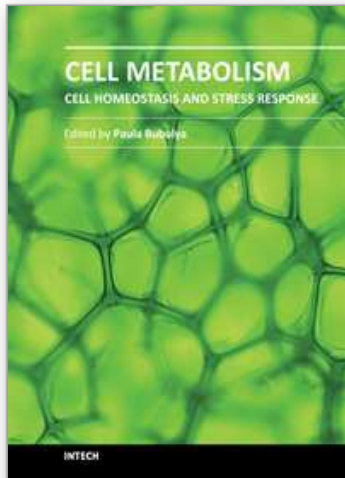
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A global research community of scientists is teasing out the biochemical mechanisms that regulate normal cellular physiology in a variety of organisms. Much of current research aims to understand the network of molecular reactions that regulate cellular homeostasis, and to learn what allows cells to sense stress and activate appropriate biochemical responses. Advanced molecular tools and state-of-the-art imaging techniques discussed in this book continue to provide novel insights into how environmental changes impact organisms, as well as to develop therapeutic interventions for correcting aberrant pathways in human disease.

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