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Photodynamic Therapy in Skin Cancer

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1. Introduction

In the last decades the improvements in cancer treatments were an important challenge both for physicians and researchers. The major aim was to obtain a targeted therapy that selectively destroys tumor cells, without affecting the normal tissues. For a very long period of time, the classical methods of treatment were surgery, chemotherapy and radiotherapy. Nevertheless, in most cases, the classical therapies are efficient only in the incipient stages and the solid tumors are often resistant to treatment. Two thirds of the patients are discovered in advanced stages of the disease. Even treated, their death occurs because of relapses and metastases. A quarter of the patients with a tumor in an operable stage can not benefit from the treatment because of their age and co-morbidities. They can only receive palliative therapy with a high relapse percentage.

The concept of cellular death determined by the interaction of light with certain drugs was developed a century ago. The first to describe this process was Oscar Raab, in 1900. von Tappeiner and a dermatologist, Jesionek, used a combination between topically applied eosin and white light for the treatment of cutaneous tumors. In fact, Hermann von Tappeiner was the one who introduced the term "Photodynamic Therapy" (PDT) in clinical use, demonstrating the necessary presence of oxygen for the photosensitizing reaction to take place. In 1942, Auler and Banzer injected hematoporphyrin in tumor-bearing animals and observed that, after exposure to a halogen lamp fluorescence, the tumor necrosis appeared. The purification of hematoporphyrin, the use of its derivatives, and especially the introduction of LASER as a light source by Maiman led to improvements in the effects of this therapy.

The modern era of PDT began in 1960 with the studies of Lipson and Schwartz, when they observed the red fluorescence of neoplastic lesions after the injection of hematoporphyrin compounds. Since then, considerable efforts have been made to improve the effects of this therapy. 5-aminolevulinic acid was used in 1990 by Kennedy and represented a major advancement in PDT. The first approved photosensitizer to be used on humans was Photofrin.

Today, PDT is a non-invasive cancer therapy that associates the use of three components: the photosensitizer (PS), the light, and oxygen. PDT consists of the administration of a PS which accumulates in the lesion and the irradiation of the affected area with light in order to activate the drug. Thus, PDT becomes a treatment with double specificity. On one hand, PS accumulates selectively in the tumor, on the other hand the irradiation is targeted at the tumor. Several factors favor the selective accumulation of PS in tumor cells, including their high proliferation rate, the increased number of low density lipoprotein receptors, the increased vascular permeability, and the production of collagen which can bind the PS (Nowis et al, 2005).

2. Photosensitizers

The main classes of photosensitizers are porphyrin derivatives, chlorines, phthalocyanines and porphycenes.

One of the gold standards for the PDT is to find the ideal PS. This should comply with some criteria: to be chemically pure; to have chemical and physical stability; to be activated only in the presence of light, with no dark toxicity; to have the absorption peak at a wavelength >630 nm, where it presents an optimal tissue penetration; to have a high absorption coefficient; to be rapidly and predominantly retained in the tumor tissue; to be rapidly eliminated from the organism, so as to prevent the risk of prolonged systemic photosensitivity; to have a clearance from the tumor tissue slower than that of normal cells; to generate cytotoxic reactive oxygen species (Gomer & Henderson, 1998). To these desiderata some principles that must be followed should be added for the effectiveness of PDT responses and depend on the localization of the PS: the wavelength of the light used for the PDT must correspond to the PS's absorption peaks; adequate time must be considered following PS administration in order to allow the substance to penetrate the targeted cells. Nevertheless, the risk of skin cancer after the UV irradiation should be taken into consideration. To minimize this risk, the wavelength of the light used for the PDT treatment must be longer than those in the UV spectrum.

None of the PS used until now complies with all these criteria and this is the reason why new PS are being studied in the search for one with superior properties.

The most studied PS are porphyrins. These are a group of pigments that have in their structure a tetrapyrrolic macrocycle and a metal ion that mediate the biological reactions of oxidation. There are natural porphyrins synthesized by live matter. One of the best known porphyrin structures is the heme ring, a tetrapyrrolic macrocycle with iron that is produced by the mitochondria with the help of an enzymatic complex formed by 8 enzymes. The synthetic porphyrins are heterocycle macrocycles characterized by the presence of some modified pyrrolic subunits interconnected at the level of carbon atoms by methyl bridges ($=CH-$) and have the hydrogen atoms substituted in the meso-position. The structure contains 18 electrons that can form bonds with metal ions (Kral et al, 2006). The first synthesized porphyrin by Rothmund in 1936 was tetraphenylporphyrin (TPP). Since then, numerous porphyrins have been synthesized, both symmetric and asymmetric ones. The study of synthetic proteins was started in 1960, and the first isomer was synthesized by Vogel in 1986, porphycene. There were synthesized porphyrins with contracted molecules, (e.g. corrole), but also expanded ones. Texaphyrins have a peak of absorption in infrared, at 730-770 nm, which allows a high penetration in the tissues and might be used in the treatment of thick tumors or of melanomas (Kral et al, 2006).

Under the influence of light, the photosensitizer passes from ground to the excited singlet state. From this phase, the PS may either decay to the ground state, or pass to the triplet excited state. From this last condition, the PS can react with the oxygen molecules in 2 ways: a direct reaction with a substrate, mediated by hydrogen or electron transfer (type I photooxidation), or energy transfer to nearby molecules of oxygen (type II). During the type I reaction free oxygen radicals are formed, while during the type II reaction singlet oxygen is formed. All the generated oxygen species (ROS) can attack the susceptible surrounding substrates. However, singlet oxygen is considered to be the main cytotoxic agent in PDT (Triesscheijn et al, 2006).

There are different criteria in order to classify the PS. According to their water solubility, the photosensitizers (PS) are hydrophobic, hydrophilic and amphiphilic. The hydrophobic PS

don't have electric charges in the periphery and are not soluble in water or alcohol. The hydrophilic PS have 3 or more peripheral substitutes that are electrically charged and they dissolve in water at a physiological pH. The amphiphilic PS have 2 or less than 2 peripheral substitutes and are soluble in water or alcohol at physiological pH (Boyle & Dolphin, 1996). According to the modality of administration, PS can be administered systematically or topically, as a prophotosensitizer.

The systemic photosensitizers include porfimer, benzoporphyrin derivative monoacid ring A (BPD-MA), metatetrahydroxyphenylchlorin (mTHPC, temoporfin) and tin ethyl etiopurpurin (SnET2). After their administration, a long-lasting photosensitivity can persist, because they are retained in tissue macrophages and tumor cells.

The prophotosensitizers have a low-molecular weight, are hydrophilic and are topically administered on the skin surface. These include 5-delta-aminolevulinic acid (ALA) and methyl-esterified ALA (MAL). In fact, the topical application of ALA became the most common used technique in dermatology (Torpe & Bhardwaj, 2008).

The most extensive way of studying PS is to describe them in the chronological manner in which they entered in use. We can describe PS of first, second, third generation. The first generation of PS includes: hematoporphyrin (HpD), hematoporphyrin derivatives and the commercial purified compound Porfimer sodium or Photofrin. Photofrin is a mixture of hematoporphyrin products with different absorption peaks and it is the active purified fraction of HpD. It was the first PS approved for PDT for recurrent, superficial papillary bladder cancer. HpD is an effective tumor killer in red light PDT. HpD was the first porphyrin used in clinical practice not only on patients with bladder cancer, but also skin cancer. At 630 nm, the wavelength most used in the clinic to activate porfimer sodium, it present a weak absorption ($1,170 \text{ cm}^{-1} \text{ mol}^{-1} \text{ l}^{-1}$). The skin photosensitivity after PDT with porfimer sodium lasts for 4-12 weeks (Triesscheijn et al, 2006). To obtain similar results with PS of the second generation, the first generation ones must be used in high quantities and with a high fluence rate. This stimulated the research for new PS (Nowis et al, 2005).

From the second generation of PS, ALA is the most used. Applied topically, it increases the concentration of protoporphyrin IX (PpIX) in the tumor cells. Actually, the PS based on ALA are not intrinsic photoactive but they accumulate preferentially in the tumor cells and are metabolized by the pathway of heme biosynthesis becoming a photosensitizing porphyrin. If the cells don't undergo PDT the porphyrines are metabolized to heme, which is photodynamically inactive for 24-48h. Protoporphyrin IX (PpIX) is a porphyrinic compound with photodynamic activity, which can be synthesized by all the nucleated cells. When activated by light, at 630 nm, PpIX emits red fluorescence. ALA is a natural precursor of heme. Two molecules of ALA form uroporphobilinogen (PBG). Four molecules of PBG form uroporphyrinogen, which is then converted into coproporphyrinogen. Inside the mitochondria, the latest is converted into protoporphyrinogen IX, and under the action of protoporphyrinogene oxidase, this is converted into PpIX. In the end, under the action of ferrochelatase, the Fe^{+2} is incorporated into the tetrapyrrol ring, and the PpIX is converted into heme. The tumor cells have a lower ferrochelatase activity than normal cells (Calzavara Pinton et al, 2007, Kondo et al, 1993). After the administration of ALA, the capacity of ferrochelatase is exceeded, and PpIX accumulates into the tumor. The accumulation of PpIX depends on the activity of ferrochelatase and the Fe^{+2} availability. The Fe^{+2} source is represented by mitochondrial reserve and the simultaneous administration of ALA and iron chelators (EDTA, desferrioxamine) increases the rate of accumulation of porphyrins. If the level of iron

decreases, the ferrochelatase may incorporate Zn in the PpIX leading to the formation of fluorescent protoporphyrines with Zn. ALA can be administered systemically (orally, intravenously, inhalatory) or topically. PpIX is detected in the epidermis within 3-8 hours after systemic administration of ALA. In general, PpIX is eliminated from the blood 24-48 hours after the topical or systemic administration of ALA. The peak in the plasma of PpIX is detected 8-12 hours after the oral administration (60 mg/bw), 4,1 hours after inhalatory administration (500 mg) and 2,9 hours after the instillation into the bladder (Rick et al, 1997). For that reason, the risks of prolonged sensitivity are minimal. After the topical application of ALA on the skin neither ALA nor PpIX can be detected in the blood stream. ALA has been approved for PDT treatment since 1999.

The use of ALA has advantages as compared to porfimer sodium. First of all, skin sensitivity lasts only 1-2 days due to the more rapid clearance. On the other hand, it can be applied both topically, for the treatment of skin cancer, but also systematically, for cancer in the oral cavity, or digestive tract, with a good selectivity for the tumor cells.

The major disadvantage of ALA is that it traverses the cellular membrane with difficulty because of its hydrophilic profile and it may accumulate with difficulty in the tumor cells. This process is dependent on energy, pH and temperature and is slow, although in the tumor cells it is more rapid than in healthy ones.

From our studies on Walker tumor in rats (Filip et al, 2008), the concomitant administration of ALA with chitosan increased the level of PpIX in tumor significantly after one hour as compared to the sole administration of ALA. Six hours after administration PpIX disappears from the plasma. The administration of chitosan favors the plasmatic clearance and the concentration in the tumor of the active porphyrin.

On the contrary, the alkyl esters of ALA can penetrate easily into the cells. MAL, the esterified derivative of ALA (methyl ester of ALA), is lipophilic and with an increased selectivity for tumor cells as compared to ALA. The transmembranary transport of MAL is made by mechanisms of facilitated diffusion, being more efficient in tumor cells. Due to their increased permeability, the selectivity of MAL for tumor cells is greater as compared to ALA. After entering the cell MAL is demethylated to ALA and the next steps to PpIX are similar. Prior to MAL topical application, the stratum corneum of the skin is removed by superficial curettage.

Second generation PS like meso-tetrahydroxyphenylchlorine (MTHPC) or benzoporphyrin derivative monoacid A ring (Verteporfin) have a limited cutaneous photosensitivity. They were administered systemically for the treatment of basal cell carcinoma and Bowen's disease. mTHPC (temoporfin, Foscan) has an absorption peak at 652 nm with a better penetration of the light in the tissues as compared to ALA and porfimer sodium. Foscan was approved in 2001 in EU for the palliative treatment of head and neck cancer (Triesscheijn et al, 2006).

In the search for newer PS numerous third generation PS were developed. These are generally activated by higher wavelengths, have an increased tumor specificity and the generalized photosensitivity is shorter. The most studied are tin ethyletiopurpurin (SnET2), mono-L-aspartyl chlorine e6 (Npe6), benzoporphyrin derivative (BPD), lutetium texaphyrin (Lu-Tex).

To improve the effects of PDT, some methods to increase the efficacy of existing PS were researched and new PS were tested. Phthalocyanines are macrocyclic compounds and have a major disadvantage for the clinical application which reduces their efficiency in PDT: they have a strong tendency to form oligomers, especially dimers. Zinc phthalocyanines' incorporation into liposomes decreases their aggregation degree (Garcia et al, 2011). In vitro

and in vivo studies have been conducted with new PS. For example, phthalocyanine-polyamine conjugates proved a high phototoxicity against human colon adenocarcinoma HT29 cells and hamster ovary cells. They have high affinity toward the lysosomes, but not the mitochondria, and they inhibit the growth of the tumor without toxicity for the normal cells (Jiang et al, 2010). N-heterocyclic (NHC) – pyridine complexes incorporating a carbene unit as an ancillary ligand were synthesized (Chen et al, 2011), chlorine compounds with conjugated substituents such as vinyl groups and carboxylic acids (Eriksson & Eriksson, 2011), cyanine HM 118. Also, chlorine e6 (Ce6) – conjugated glycol chitosan nanoparticles seem to have potential for PDT (Lee et al, 2011) as well as tumor-targeting albumin nanoparticles containing Ce6 (Jeong et al, 2011). The use of nanomaterials as carriers for the PS tends to improve some of the PDT disadvantages. For example, a folic acid conjugated graphene oxide loaded with Ce6 can accumulate in the tumor cells and shows a great potential for PDT (Huang et al, 2011). Also, glycoconjugated fullerene has a potential use in PDT (Otake et al, 2010).

3. Light sources

An efficient PDT requires not only the appropriate delivery of the light from the source to the target, but also a homogeneous light distribution. The intensity of the irradiation should be verified at the treated point using light detectors. There are four kinds of detectors used in optics: thermopile needed for the measurement of temperature, pyroelectric detector that measures the electric current produced by a crystal when its temperature is modified, photomultiplier tube and the integrative sphere (Sibata et al, 2000). The ideal irradiation during PDT is given by the optimization of the distribution of the light so that is appropriate for its geometric distribution of the PS and matches the optical properties of the targeted tissue so that the effects on the neighboring zones are minimal. A good evaluation of the effect of the PS can be made by dosing the concentration of PS in target tissue. The ideal dosimetry optimizes the distribution of the light dose to the treated volume by selecting the best geometry for irradiation (Jacques, 1998). Techniques of measuring the quantity of PS in vivo non invasively by using elastic scattering were developed (Mourant et al, 1999). The types of interaction of light to the tissue depend on the wavelength and the properties of the irradiated volume. There are five types of interactions: photochemical, thermal, photoablation, plasma induced ablation and photodisruption. For the clinical use of PDT only the photochemical and sometimes thermal interactions are important. It was shown that PDT is synergic to sub-lethal hyperthermia (Chen et al, 1996). In preclinical trials PDT with low power was used to avoid the thermal interactions.

The PDT wavelength ranges between 600 and 1000 nm. Increased wavelength correlates with increased tissue penetration. The minimum threshold was established at 600 nm because hemoglobin absorbs under 600 nm. The penetration depth is given by the thickness of the tissue where the intensity of the incident light decreases by half. It is at about 2-3 mm for 630 nm and increases to 5-6 mm for 700-800 nm. Endogenous chromophores, such as melanin, can interfere with the PS for the absorption of light. This is important in the treatment of patients with a dark phototype or when treating the hyperpigmented lesions of metastatic melanoma.

In PDT red light is generally used (580-700 nm) in a dose of 100-150 J/cm², and the intensity of the light dose does not surpass 200 mW/ cm² to avoid the hyperthermic effects (Clark et al, 2003). For inflammatory skin disorders red light in a dose of 10-40 J/cm² and an intensity

of 50-70 mW/cm² is used in more than one session (Babilas et al, 2006). Porphyrins exhibit typical absorption with the highest peak at 405 nm, called the Soret-band. There are also several Q-bands, the last having the absorption peak at 635 nm, this wavelength is used for irradiation in PDT. It is also possible to use blue light in combination with 5-ALA hydrochloride (Levulan) for the treatment of actinic keratosis.

Visible light from 2 types of light sources is used: noncoherent, conventional or coherent (laser).

The incoherent, broad spectrum light sources are simple, cheap and easy to use both for in vivo and in vitro studies. Such devices are halogen, tungsten, xenon or fluorescent lamps. To select the different wavelengths they are used together with optical filters. Their disadvantages are represented by an important thermal effect, the low light intensity and the difficulty to control the dose of light.

Light emitting diodes (LED) are also incoherent sources available for PDT. LED may generate high energy light with the needed wavelength. LED with a large surface are used for irradiating extended lesions. The usual doses are 37-50 J/cm² and the power output is up to 150 mW/cm².

LASERS emit a precise wavelength, monochromatic, with high power but are expensive and need high level technical support (Triesscheijin et al, 2006). An important development in PDT is the availability of LASERS with diodes at wavelengths compatible with the most used PS (Wilson, 1998). This system has a low electric power and a cooling system similar to that of a LASER. The advantage of diode LASER is the low cost of acquisition and maintenance, small dimensions and portability (Sibata et al, 2000).

4. Mechanisms involved in PDT effects

The mechanisms involved in PDT's effects are very complex and not entirely understood. The key effect achieved using PDT is the damage of the tumor cells; this can be obtained both directly and indirectly. The direct antitumor effect implies the cellular destruction by apoptosis or necrosis. The indirect effects of PDT consist, on the one hand, in the stimulation of the immune response of the host and, on the other hand, in the damage produced to the vasculature, with consecutive disruption in the tumor micromedium. The contribution of each mechanism in the occurrence of cell death is hard to be established and depends on the type of photosensitizer (hydrophilic or hydrophobic), the light fluence rate, the timing of illumination following photosensitizer administration, the depth of the tumor localization. The type of photosensitizer influences its subcellular localization and the organelles that are primarily damaged. The lipophilic PS enter the cell through receptor mediated endocytosis (Marcus & McIntyre, 2002) and accumulate in the lipophilic compartments of the cell: plasma, mitochondria, endoplasmic reticulum, cellular membrane and lysosomal ones (Schneider et al, 2005). The effects appear a few hours from the treatment and they include: the inactivation of the membrane enzymes, the increase in its permeability, the formation of vesicles, the ceasing of the cellular respiration and the cellular death through apoptosis or necrosis. The hydrophilic PS administered i.v. bind to the plasma proteins and enter the tumor tissue. Due to the fact that they have a reduced capacity to traverse the cellular membrane they will mainly affect the tumor vasculature leading to ischemia and consecutively hypoxia (Canti et al, 2002). Another modality of modulating the effects of PDT is through the light dose and the photosensitizer used. When low doses of PS are used, the cells remain viable, but the intracellular signaling pathways, the cytokine formation and the

receptor expression are altered. When using high doses of light and/or PS cellular necrosis is triggered through the lesions in the membrane of the cell and organelles, with the subsequent formation of an inflammatory status. When using intermediate doses apoptosis is favored. The apoptotic process is mainly triggered by PS that accumulate preferentially in the mitochondria such as phthalocyanines, porphycenes, verteporfin, hypericin (Calzavara-Pinton et al, 2007), Photofrin, protoporphyrin IX (Kessel & Luo, 1999).

4.1 The effects of PDT on ROS

As described before, under the influence of light, the PS lead to ROS generation, the main cytotoxic agent being singlet oxygen. It has a short life (30-180 nanoseconds) and it diffuses on short lengths (<50 nm) (Niedre et al, 2002), so that, in the end, the localization of the PS in the cell will dictate the cellular structure that will become a target for the singlet oxygen (Peng et al, 1996). For example the PS which will incorporate into mitochondria will determine Bcl-2 lesions that will induce the apoptosis of the tumor cells. Foscan fixes in the endoplasmic reticulum inactivating cytochrome oxidase -NADPH and also in the Golgi apparatus inactivating UDP-galactosyltransferase (Teiten et al, 2003).

It seems that the singlet oxygen can interact with more than one cellular structure. Firstly, singlet oxygen can attack the cellular membrane lipids leading to the formation of lipids hydroperoxides, that can initiate the oxidation chain of unsaturated lipids and continue to determine effects on other cellular structures (Gutteridge & Halliwell, 2000). Secondly, the membrane and plasma protein may become the target of the toxicity mediated by singlet oxygen with the modification of the amino acid chains especially at the level of cysteine, histidine, methionine, tryptophan and tyrosine (Schafer & Buettner, 1999). The modification of tyrosine as result of the interaction with ROS is highly important leading to the formation of the tyrosyl radical which dimerises and forms dityrosine (Pfeiffer et al, 2000). Tyrosine is normally involved in the intracellular signal transduction on the tyrosine phosphorylation pathway. The oxidation of tubulin is also important because it leads to lesions of the microtubules determining their inactivation and this makes the photosensitized cells unable to divide blocking the cell cycle in the G2/M phase (Berg & Moan, 1997).

The photo-oxidation of lipids and proteins in the cellular membrane activates the membrane phospholipase leading to the degradation of the altered phospholipids, the modification of the membrane fluidity and the loss of cellular integrity. The alterations affect the membrane enzymes but also the cellular receptors. The membrane depolarization and the ionic equilibrium are also altered.

The effects of ROS manifest themselves also at the level of the mitochondrial and lysosomal membranes. Apoptosis as a result of PDT is a consequence of the release of cytochrome c, the apoptosis induction factor and activation of caspase (Pogue et al, 2001; Rancan et al, 2005). The attack of singlet oxygen at the level of the lysosomes leads to the release of the lysosomal enzymes which determines extensive cellular damage leading to a late apoptosis and/or relocation of the sensitizer in another area of the cell. On the other hand, the effects upon the DNA are rare and the mutagenicity seems to be reduced (Ben-Hur et al, 1987).

Photodynamic therapy induces oxidative stress but also the release of nitric oxide (NO) in the tumor area (Hirst & Flitney, 1997). It determines vasodilatation and increases the blood flow to the tumor facilitating tumor growth and metastasis (Gomes et al, 2002). The tumors that generate low amounts of NO are susceptible to PDT as compared to those that generate high ones. The production of a high amount of NO stops the inflammatory reaction that

arises after PDT and also it blocks the effects seen normally after PDT on the blood flow such as vascular occlusion and ischemia. These phenomena are explained by NO capacity to prevent platelet aggregation and endothelial adhesion, and to stop the neutrophil accumulation. NO also inhibits the expression of adhesion molecules and mast cell degranulation (Korbelik et al, 2000). NO has a double function. During treatment it determines vasodilatation and favors ROS generation and after treatment it favors the formation of ischemia/reperfusion lesions, cell apoptosis and the immune reaction with an antitumoral effect (Dougherty et al, 1998; Korbelik et al, 1998).

NO has a cytoprotector effect through a mechanism dependent of cGMP. The protection of tumor cells is realised by inhibiting caspase activation by S-nitrosylation (Gomes et al, 2002) and the modulation of the gene expression through a cGMP dependent mechanism or S-nitrosylation. NO blocks the lipid peroxidation chain by the neutralization of ROS generated by PDT. NO is a scavenger of the superoxide anion but also of the alcoxyl and peroxy radicals (Rubbo et al, 1994). Through the reaction of NO with the superoxide anion the peroxynitrite anion is generated (ONOO⁻), which decomposes to form strong oxidants that can provoke tissular damage. Once more NO has a dual role being at the same time a cytoprotector and an aggressor for the cells. It is possible that NO and iNOs have a role in the induction of apoptosis by disturbing the membrane potential and increasing the permeability of the mitochondrial membrane (Thor et al, 1985).

The photodestruction determined by ROS may be partially prevented by the antioxidant enzymatic system: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). It seems that the activity of SOD increases during PDT as a result of ROS generation in the cell (Saczko et al, 2007), therefore SOD is considered to be an inducible antioxidant enzyme that regulates the sensitivity of tumor cells to PDT (Huang et al, 2000). Nonetheless it seems that just MnSOD is important for the cell response to PDT and the alteration of the mitochondrial function is a critical factor for phototoxicity so that MnSOD may be a molecular target that could modulate the cell sensitivity to PDT (Haylett et al, 2003). The inhibition of SOD activity in tumor cells potentiates the PDT cytotoxic effect and the transitory transvection of MnSOD gene but not the CuZnSOD gene reduces the efficiency of PDT.

The studies made on an experimental model of Walker carcinosarcoma (Daicoviciu et al, 2008), showed that PDT with 5-ALA determines a decrease in the antioxidant enzymes from erythrocytes especially CAT and GPx and the activity of MnSOD increase in tumor homogenates. The behavior of erythrocyte and tissue SOD is adaptive as a result of post-PDT ROS generation. The reduction of the antioxidant enzymes from the erythrocyte after PDT is explained by the lesions in the erythrocyte's membrane and the alteration of the structural protein under the influence of ROS (El-Missiry & Abou-Seif, 2000).

4.2 The effects of PDT on angiogenesis and extracellular matrix

The lesions to the vascular endothelium are part of the indirect mechanisms by which PDT can determine the destruction of tumor cells. The plasmatic concentration of the PS may be a good indicator of the PDT efficiency (Triesscheijin et al, 2006). The complete obliteration of the vessels that feed the tumor is essential for long term good results. PDT damages the cytoskeleton of the endothelial cells (Chaudhuri et al, 1987; Sporn & Foster, 1992), it increase the adhesion and activation of platelets, the secondary release of eicosanoids, especially tromboxane that mediates vasoconstriction and the formation of thrombus (Fingar, 1996). The

damage to the endothelial cells leads to the release of leukotrienes which increase the cellular permeability and the interstitial pressure determining consecutive vasoconstriction. Together, the vasoconstriction, the high interstitial pressure and the thrombus lead to ischemia and tissue necrosis. The phenomena are extremely complex because the tissue hypoxia is a triggering factor for tumor angiogenesis. So PDT may be considered proangiogenic because the oxidative stress and the hypoxia consecutive to PDT may activate angiogenic growth factors and an inflammatory adaptive response which, in turn, will initiate tumor angiogenesis (Dougherty et al, 1998). In the meantime, the extracellular matrix (ECM) serves as structural support and provides informational guidance for developing vasculature.

Matrix metalloproteinases (MMPs) are a heterogeneous group of endopeptidases that are synthesized in a latent state. Their activation through photolytic cleavage of $-NH_2$ terminal prodomain was correlated with the mechanisms involved in PDT. Cytokines, growth factors, oncogenes and, also, ROS are potent factors involved in the activation of MMPs (Overall & Lopes-Otia, 2002). In return, the activity of MMPs on growth factors, chemokines, growth factor receptors, adhesion molecules and apoptosis mediators is essential for the rapid cellular responses critical for angiogenesis and is also involved in mediating tumor growth and progression (Coussens et al, 2002). A large variety of cells, including endothelial cells, fibroblasts, inflammatory cells, can produce MMPs (Werb, 1997). It appears that MMP-2 and MT-1 MMP are essential for the tumor angiogenesis. MMP-2 together with MMP-9 is part of the initial phases of tumor angiogenesis, for the formation of the microvasculature and in the late stages of the process, for the involution, regression, and re-absorption of the neo-vasculature. The most important functions of MMP-2 and MMP-9 are the proteolysis of EMC, the proteolysis of the cell surface the release of growth factors and the activation of cytokines/chemokines (Davidson et al, 2003). The MMPs activity is modulated by the tissular inhibitors (TIMP), an unbalance between the expression or activity of MMPs and TIMP leading to tumor growth. PDT increase the MMPs expression in tumors and the association with MMPs inhibitors increases the therapeutic effect (Ferraio et al, 2004). There may be a connection between the inflammatory process after PDT, as it will be discussed, and the activation of MMPs, one proof being the increase in expression of MMP-1 and MMP-3 dependent on $Il-1\alpha$ released from the fibroblasts exposed to 5-ALA PDT (Karrer et al, 2004). $Il-1\alpha$ is a good NF- κB inducer and it seems that this transcription factor may modulate the activity of proteases (Matroule et al, 2006).

From our own experience regarding 5-ALA PDT on Walker carcinosarcoma, we observed the increase of MMP-2 activity in the tumor 1 hour after PDT. The association between chitosan and 5-ALA in biocomposite modifies the effect of MMP-2, the chitosan increases the curative effect of the antitumoral treatment probably by improving the host immune response (Filip et al, 2008). On the same experimental model PDT with 5,10,15,20-tetrasulfonato-phenylporphyrina (TSPP), showed the increase in MMP-2 activity 3 hours after the treatment. The increased values were also observed 14 days after treatment (Clichici et al, 2010).

At the level of the extracellular matrix, PDT also affects the proteoglycans, which have a role in the integrity of ECM (Lopes et al, 2006). Studies regarding this issue are still relatively few. The decrease of versican, a proteoglycan involved in the cellular proliferation was highlighted after PDT with methylene blue (Wight, 2002). At the same time it seems that PDT has effects on ICAM-1 (the intercellular adhesion molecule 1), VCAM-1 (the adhesion molecule to vascular cells), PECAM-1 (the adhesion molecule of the platelet to the endothelial), these adhesion molecules having as a result the extravasations of circulating

tumor cells and metastasis (Kobayashi et al, 2007). The results are contradictory, some studies showed a decrease in the ICAM-1 and VCAM-1 expression after PDT (Volanti et al, 2004), while other studies showed the increase of ICAM-1 expression on the surface of leucocytes in tumors treated with PDT (Castano et al, 2005).

One of the most important regulators of angiogenesis is the vascular endothelial factor (VEGF). Among the factors which determine the release of VEGF from the hypoxic cells are MMPs (Lee et al, 2005). VEGF promotes the proliferation, migration and survival of endothelial cells through action on the VEGFR-2 receptor, but also the secretion of some specific tissue factors through action on VEGFR-1, among which is the tissue plasminogen activator, urokinase, growth factors, and MMP-9 (Ferrara, 2004). The over-expression of VEGF associates with the progression of the tumor which leads to poor prognosis for the patients (Way et al, 2004). That is why combining anti-angiogenic therapy with special irradiation techniques, for example high-frequency ultrashort pulsed laser, can represent a progress as a vasculature-disrupting therapeutic modality for cancer treatment (Choi et al, 2011)

4.3 PDT and immunity

An important desiderate for an antitumoral therapy is to increase the capacity of the host immune system to recognize and destroy tumor cells, including the residual post-therapy cells, both at the site of the tumor and at distance (Castano et al, 2006). PDT can activate or suppress the immune system depending on the type of PS and the light dose. It seems that PDT activates both the non-specific inflammatory system and the specific one, with the involvement of the antigen presenting cells and lymphocytes (Korbelik, 1996, 2006). The degree of the immune response of the host depends on the chemical nature of the PS, its concentration and subcellular localization, the characteristics of the light source, light fluence and fluence rate, oxygenation level and tumor type (Firczuk et al, 2011).

The tumor treated with PDT becomes massively infiltrated with cells, mostly neutrophils mast cells and macrophages. In the mean time it increases the proinflammatory cytokines IL-1 β , TNF- α , IL-6, the macrophages inflammatory proteins (MIP-1, MIP-2) and the cell adhesion molecules E-selectin and ICAM (Dougherty et al, 1998).

The neutrophils accumulated in the vasculature of the tumor, destroy the vascular endothelium, release chemotactic factors for other inflammatory cells and also oxygen metabolites with a role in damaging the tumor tissue. The activated neutrophils play also an important role in the long term suppression of tumor growth. In the initial phases, after PDT, the activation of phagocytosis or the massive destruction of the tumor cells, creates optimal conditions for macrophages, dendritic cells and the antigen presenting cells to process the tumor antigens allowing the specific antitumoral immunity to arise (Krosi et al, 1996). The bigger the amount of cellular detritus the better the processing of the tumor antigens by macrophages (recruited at the tumor site) is facilitated with the recognition of the specific epitopes and the activation of lymphocytes (LT), capable to eliminate the remaining cell after the treatment (Hunt & Chan, 1999).

The macrophages are considered by some authors (Korbelik, 1996) to play a central role in the immunological changes that occur after PDT because they have a tumoricid effect and are also involved in the release of pro- and antiinflammatory cytokines. The association of PDT with granulocyte-macrophage colony-stimulating growth factor enhances the effects of PDT (Krosi et al, 1996).

As we have already discussed, PDT will determine a specific immune reaction with an important role in the long term control of the tumor development, a type of immunity that resembles to that obtained after bacterial vaccines (Korbelik & Dougherty, 1999). The antigen presenting cells will process the tumor antigens and will present them on the surface of the membrane together with the major histocompatibility complex class II. This is followed by the activation of LT helper and consecutively cytotoxic LT. CD4 and CD8 clone T cells are formed, they are capable of recognizing the tumor cells, they expand rapidly and get activated and generate a strong immune response (Van Duijnhoven et al, 2003). In addition the LB and NK cells are activated contributing to the immune response after PDT. PDT can modulate the expression of IL-6 and IL-10 in the normal tissue and the tumor one mediated by the transcription of AP-1 (Dougherty et al, 1998). IL-6 has an important role in PDT because it inhibits the cellular proliferation and by this it potentiates the therapeutic effect of PDT. Blocking the function of this cytokines decreases the effect of PDT (Dougherty et al, 1998). IL-6 also has an inductor effect on the Th-17 subset of lymphocytes that produces IL-17 a cytokine with a role in the amplification of the production of chemokine, proinflammatory cytokines and matrix metalloproteinases capable of mediating the infiltration and destruction of the tissue (Wei et al, 2007). In addition, the hyperproduction of IL-6 accentuates the effect of PDT of inhibiting cell proliferation by perturbing the cell cycle progression at G1/S check point (Ahmad et al, 1999). Nevertheless TNF- α , IL-1 β , IL-2, IL-6, IL-8 and IL-10 may represent criteria of evaluation for response to PDT (Yom et al, 2003). PDT amplifies the host immune response; this may explain the regression of untreated tumor metastasis after PDT applied on the primary tumor (Van Duijnhoven et al, 2003). Because lymphocytes act not only on tumor cells from the primary site but also on the metastasis, PDT may be considered a systemic therapy.

The immune response after PDT cannot be discussed without talking about the NF-kB factor. There are two pathways of activating NF-kB namely the classical pathway and the alternative one (Bonizzi & Karin, 2004). The reactive oxygen species generated by PDT lead to the activation of NF-kB, the first observations regarding this phenomenon were made on leukemia cells L1210 treated with Photofrin (Ryter & Gornerr, 1993). In 1995 it was observed that using methylene blue as a PS on monocytes and lymphocytes latently infected with HIV-1 it increases the production of singlet oxygen and the activity of NF-kB leading to the activation of the virus (Matroule et al, 2006). It seems that NF-kB has a primordial role in recruiting neutrophils during PDT and it favors the apoptosis in lymphocytes by expressing the Fas ligand (Lin et al, 1999). It also has an antiapoptotic role by promoting the expression of genes that protect against apoptosis (Karin & Lin, 2002). It was observed that NF-kB mediates COX-2 expression; the inhibition of these enzymes improves the response to PDT (Matroule et al, 2006).

In conclusion, the inflammatory reaction is a response that is due to the cytotoxic effects exerted on the tumor cells by PDT, but also due to the vascular changes with the release of vasoactive and proinflammatory mediators and in the meantime the induction of signaling cascades and transcription factors that trigger secretion of cytokines, MMP2 and adhesion molecules (Gollnick et al, 2003; Firczuk et al, 2011).

4.4 PDT effect on COX-2

The cyclooxygenase 2 (COX-2) catalyzes the conversion of the arachidonic acid into prostaglandins and it is an important mediator of angiogenesis due to the production of VEGF and the stimulation of sprouting, migration and formation of vascular tubes (Gately

& Li, 2004). In PDT with photofrin it was shown an increase in the expression of COX-2 (Ferrario et al, 2002). The prolonged administration of COX inhibitors before irradiation increases the response to the antitumoral therapy (Makowski et al, 2003). The COX-2 inhibitor may act as an angiogenic factor which enhances the effects of PDT. The expression of COX-2 increases 30 minutes after PDT and it remains at the same level 12 and even 72 hours. It seems that the oxidative stress generated after PDT activates a cascade of protein kinases including p38, with a role in increasing the expression of COX-2. The molecular mechanisms that modulate the expression of COX-2 in tumor cells involve the NF- κ B dependent transcription of the COX-2 gene, without the involvement of other mechanisms of post-transcriptional regulation (Hendrickx et al, 2003; Volanti et al, 2005).

4.5 The cytotoxic effect of PDT

Apoptosis is a type of cellular death encountered most frequently in PDT because most of the PS accumulate in the mitochondria (Kessel & Luo, 1999). It appears quickly, at about 30 minutes after the photolesion (Oleinick et al, 2002). When the PS is located outside the mitochondria or high doses of PS are given, the type of triggered cellular death is necrosis (Almeida et al, 2004).

Two types of apoptotic process are being described, one is mediated by the mitochondria (the intrinsic pathway) and the other is receptor mediated (the extrinsic pathway) (Almeida et al, 2004). The malignant cells manifest a defective apoptosis; this explains their resistance to chemotherapy (Ahmad et al, 1999). Nonetheless PDT is efficient in the tumors resistant to cytostatics. PDT apoptosis may be influenced by other intracellular signaling pathways, including calcium, ceramides and MAP-kinases.

4.5.1 The mitochondria mediated apoptosis

The first event taking place on the intrinsic pathway of apoptosis is the interruption in the membrane potential and the release of the c cytochrome in the cytosol by opening the high conductance channels from the mitochondrial membrane or the so called mitochondrial permeability transition pore (MPTP). This is a big protein complex formed from at least three transmembranary subunits.

On tumor cell lines of human epidermoid carcinoma A 431 the administration of cyclosporin A and trifluoperazine, potent inhibitors of the MPTP opening, prevents the loss of the mitochondria transmembrane potential and the release of cytochrome c (Lam et al, 2001). The mechanism by which PDT opens the MPTP is not completely understood but it seems that a subunit of MPTP named adenine nucleotide translocator (ANT) is the target of PDT (Belzacq et al, 2001). There are data that support the association between MPTP and other proteins such as Bax, Bcl-2 and enzymes involved in the energetic metabolism such as the mitochondria hexokinase and creatine kinase.

There are also data that support the hypothesis that apoptosis mediated by mitochondria is independent on the opening of the MPTP because in some experimental models using PDT with Hypericin or Hypocrellin, the administration of inhibitors of MPTP did not prevent the changes in the transmembrane mitochondrial potential (Chaloupka et al, 1999). Some authors state that the release of the cytochrome c may be due to the peroxidation of the cardiolipin from the mitochondria (Kriska et al, 2005). The cytochrome c once released into the cytosol is bound by Apaf-1 and procaspase-9 forming a complex called the apoptosome. This self activation leads to the cleavage of caspase 9 that in its turn activates procaspase 3.

The activation of caspases 2, 3, 6, 7, 8 determines the cleavage of cellular proteins, the fragmentation of the DNA and cell death (Almeida et al, 2004).

The family of Bcl-2 proteins regulates the apoptosis induced by stimuli that act especially at the level of the mitochondria. The proteins with an antiapoptotic role from this family include Bcl-2, Bcl-xL, Bcl-w, Mcl-1, Al and Boo and the ones with a proapoptotic role Bax, Bad, Bok, Bcl-xS, Bak, Bid, Bik, Bim, Krk and Mtd (Antonsson & Martinou, 2000). In the absence of a proapoptotic signal this protein family is sequestered by elements of the cytoskeleton or by other proteins in the cytoplasm. Members of the Bcl-2 proteins are present in the mitochondria membrane, in the nuclear envelop and the endoplasmic reticulum (Gross et al, 1999). The cleavage of Bcl-2 inactivates the protein and promotes apoptosis (Oleinik et al, 2002). Members of the Bcl-2 protein family may initiate apoptosis and favor the release of cytochrome c or inhibit the apoptosis through Bid and Bcl-xL protein that in their turn inhibit the activation of caspase (Gross et al, 1999; Reed, 1998). The rapport between the proapoptotic and antiapoptotic proteins controls the cell death or survival (Adams & Cory, 1998).

Cathepsins are proteases released from the lysosomes that can cleave Bid and initiate apoptosis by releasing proapoptotic proteins from the mitochondria or cleave caspase 3 and by this blocking apoptosis. Lesions to the endoplasmic reticulum after PDT determine the release of calcium ions that may initiate apoptosis (Oleinick et al, 2002).

Data regarding the role of the Bcl-2 family in post-PDT apoptosis are controversial. Some authors state that Bcl-2 promotes apoptosis and others that it has no effect on apoptosis (Klein et al, 2001). It seems that the over-expression of Bcl-2 perturbs the mitochondrial transmembrane potential and as a consequence it affects the release of cytochrome c into the cytosol. This phenomena may be explained by the increase in the expression of the proapoptotic protein Bax in the cells that over-express Bcl-2 (Nowis et al, 2005). PDT determines a decrease Bcl-2 without affecting Bax, this rendering the cells more sensitive to apoptosis (Kim et al, 1999). It seems that Bcl-2 protein is directly altered by PDT. In the activation of caspase an important role is attributed to singlet oxygen which is produced in high quantities during PDT. The singlet oxygen scavengers such as alpha tocopherol and L-histidine inhibit the lipid oxidation after PDT and also inhibit the activation of caspase. In this respect in the PDT experiments on A 431 cell line pre-incubated with or without alpha tocopherol and L-histidine because the singlet oxygen was scavenged, the caspase-3 was inhibited (Chan et al, 2000). Also, the generation of singlet oxygen is necessary for the activation of JNK in skin fibroblasts (Klotz et al, 1997), proving that singlet oxygen is a common mediator for JNK activation in the cells that undergo oxidative stress due to variations in micromedium. It is not well understood how JNK is activated, but a possible scenario could be: JNK is phosphorylated and activated by SEK 1, that in its turn may be phosphorylated and activated by MEKK 1, the latter being controlled by singlet oxygen. This JNK activation mechanism is not mandatory for cells to enter apoptosis; the apoptotic process may be initiated by an extrinsic mechanism mediated by Fas or dexamethasone (Assefa et al, 1999; Lenczowski et al, 1997).

4.5.2 Receptor mediated apoptosis

The cellular death through apoptotic mechanisms mediated by receptor appears when the PS affects the cellular membrane of the targeted cells and it determines the polymerization of the receptors from the cellular membrane, especially the receptors belonging to the TNF

super-family. In fact, there are apoptotic mechanisms initiated by TNF on the one hand, on the other hand by the Fas-Fas ligand, but both of them involve the family of TNF receptors coupled with the signals of the extrinsic pathway. TNF is a cytokine produced especially by macrophages and it represents the major extrinsic pathway of apoptosis. For TNF there are two receptors, R1 and R2, that initiate the activation of caspase using the following membrane proteins: TRADD (tumor necrosis factor receptor type 1-associated Death Domain) and FADD (Fas-Associated protein with Death Domain). The binding of TNF to the receptors leads to the activation of transcription factors with a role in the survival of the cell and its inflammatory response. The Fas receptor also known as Apo-1 or CD 95 is a trans membranary protein that is a part of the TNF family. After its binding to the Fas ligand (FasL), a complex signal inductor of cellular death named DISC that contains FADD, caspase 8 and 10 is formed. In some cellular types (type I) the activation of caspase 8 leads to the activation of a cascade of caspase and the initiation of apoptosis. In other types of cells (type II) the Fas-DISC complex contributes to the release of proapoptotic factors from the mitochondria and finally to the activation of caspase 8 (Chen & Goeddel, 2002).

The caspase 8 might play the role of a trigger for the intrinsic pathway for PS that bind to the membrane. The inhibition of caspase 8 reduces the release of cytochrome c and the activation of caspase 3 in cells photosensitized with Rose Bengal (Zhuang et al, 1999). This effect is probably mediated by the Bid protein that is activated through proteolysis by caspase 8 and promotes the efflux of cytochrome c from the mitochondrial membrane.

5. Clinical results

PDT went beyond the stage of experiments. MAL is approved for use in Europe and United States in combination with red light for treating actinic keratosis (AK), superficial and nodular basal cell carcinoma (BCC) and in-situ squamous cell carcinoma (SCC) or Bowen's disease. A combination of an alcohol-containing ALA solution in a special applicator (Levulan Kerastick) and blue light is also approved in United States for the treatment of AK. The range of possible indications is expanding continuously, including non-malignant conditions and even premature skin aging due to sun exposure. For therapy of multiple lesions or in immunodeficient patients, PDT may be the first choice (Kalka et al, 2000). PDT is also indicated for patients with important comorbidities when surgery and radiotherapy are contraindicated. It may be used for palliative care in combination with chemo or radiotherapy for advanced tumors with skin metastases.

PDT in the dermatological pathology has some clear advantages, as compared to the conventional treatments: radiotherapy, chemotherapy and surgery. Maybe the most valuable advantage of PDT is represented by the limited duration of the treatment. In the majority of cases, one dose of PS followed by one irradiation are needed. In comparison, the radiotherapy must be performed daily for more than one week, the chemotherapy may take months, and surgery, although it consists of one procedure, the hospitalization period is long. To this, the cost-efficiency relation is added, PDT being a low cost therapy but with increased efficiency. The fact that it is a local treatment, at the level of the lesion, without affecting the surrounding healthy tissue is highly important. At the level of the lesion, necrosis may appear, but the tissue regeneration is adequate, because the collagen and elastin fibers are not destroyed. The cosmetic results are excellent. Not to be forgotten the possibility of applying the therapy again on the same region in case of recurrence, which is highly difficult when using the other classical therapies.

The side effects of PDT are relatively scarce. An important side effect is prolonged generalized photosensitivity, which led to the development of local application of PS. As a local side effect we can mention erythema, mild edema and pain. A stinging or burning sensation can occur, and that may influence patient's compliance. After a few days crusts and superficial erosions may appear and very rare, ulcerations. To reduce the pain, general anesthesia may be used, most often when treating children or extensive lesions, or local anesthesia and also premedication with opioids. However, the latter proved inefficient (Itkin & Gilchrest, 2004; Oseroff, 2005).

The results of PDT are estimated by the disappearance of the tumor, this occurs normally after 2-3 days. The result is confirmed by histopathological and cytological examinations. A partial regression is registered when only 50% of the tumor volume is reduced or when the tumor is not clinically visible but the histopathological and cytological examinations are positive. The therapy is considered to have failed when the tumor decreases less than 50% or when the patient does not present any local modification.

5.1 Applications for PDT

A particular application is the use of porphyrins for diagnosis in dermatology. After the systemic or topic administration of PS, the damaged tissue is irradiated with blue light and, due to the fluorescence of the substance, a clear delimitation from the normal tissue is observed. This fluorescent detection allows the guided biopsy of the tumor and also the complete resection of the tumor (Szeimies et al, 2005).

5.1.1 Basal cell carcinoma

Numerous studies have been conducted in order to assess the possible benefits for the use of PDT in BCC from different points of view (Richard et al, 2007). First of all, the efficiency of the therapy was taken into consideration. In a comparative study, MAL-PDT versus placebo, conducted on 66 patients with nodular basal cell carcinoma, a complete remission was observed 6 months after 2 PDT sessions in the treated group, as compared to placebo group (Foley, 2003). Other studies, with a follow-up of the patients ranging from 1 to 36 months, also reported a clearance rate for PDT in superficial BCC up to 100% (Marmur et al, 2004).

When studying the long term effects of PDT with MAL on 350 basal cell carcinoma it was observed that, after a previous curettage of the lesions and incubation with MAL for 3 hours followed by irradiation in a dose of 50-200 J/cm², the tumors were completely cured in 79% of the cases (monitored for 2-4 years) with excellent or good cosmetic results in 98% of the cases (Soler et al, 2001). Second, the efficiency of the PDT was compared to that obtained through classic methods. For the superficial BCC, PDT leads to remission rates comparable to cryotherapy, at 48 months follow-up (22% for PDT, 19% for cryotherapy), as well as at 60 months follow-up (75% for PDT, 74% for cryotherapy) (Lehmann, 2007). The results of 2 sessions of PDT with MAL for superficial BCC were compared to surgical excision in 196 patients. Three months after the therapy, the remission rates were 87,4% for the MAL-PDT and 89,4% for surgical excision (Klein et al, 2008). However, when regarding the recurrence rates, PDT seems to be inferior to surgery. An open, multicenter, randomized study which compared the effects of PDT with MAL to surgery on 101 patients with nodular basal cell carcinomas found a similar cure rate at 3 months (91% versus 98%), but the recurrence rate was significantly different (10% at 24 months for PDT and only 2% for surgery) (Soler et al.

2001). A 5-year follow-up study compared the recurrence rate after PDT with MAL and surgical excision in 97 patients. The recurrence rate was 4% for the surgery group and 14 % for MAL-PDT (Rhodes et al, 2007). The cosmetic result is superior for PDT as compared to surgery and the healing period is shorter. Because BCC have a predilection for the head and face, this could be a significant factor in choosing the modality of treatment.

During a Phase III trial, when comparing the effects of PDT with ALA 20% and cryosurgery on 88 unique superficial and nodular BCC, a clinical recurrence of 5% for ALA PDT and 13% for cryosurgery was observed 3 months after the treatment. The histological evaluation showed a 25% recurrence in the group treated with ALA PDT, and 15% for the group treated with cryosurgery (Wang et al. 2001).

This result draws attention to the insufficient penetration of topical ALA in the profound dermis. In one study, although 69% of the topically ALA-treated tumors, including BCC, SCC in situ and invasive SCC were clinically cured, only 46% of the treated tumors were histologically negative, emphasizing the possibility of recurrence (Lui et al, 1995). Improvements regarding this issue can be obtained through the curettage of the tumors right before the application of ALA or through pretreatment with dimethylsulfoxide (DMSO) or by increasing the incubation time of ALA to 48 hours or through intralesional injections with ALA. These protocols have demonstrated improvements in long term curative effects. Also, for the same purpose, the administration of ALA can be combined with ethylenediaminetetraacetic acid (EDTA) or desferrioxamine to increase the formation of PpIX (Torpe & Bhardwaj, 2008).

In nodular BCC, surgical excision remains the treatment of choice. PDT should be reserved for the patients who cannot undergo surgical therapy (Braathen et al, 2007).

For example, in a multicenter, prospectively not controlled study which included patients with recurrent superficial and nodular basal cell lesions, with a risk of complications, with previously poor cosmetic results, it was found that after two PDT sessions carried out in a one week interval the clinical remission at 3 months was 92% for the superficial forms and 87% for the nodular ones. Histological negative margins was obtained in 85% of the superficial basal cell carcinomas and in 75% of the nodular ones (Horn et al, 2003).

ALA-PDT can be used as adjuvant therapy in Mohs microsurgery for the BCC after the excision of the tumor. Applied peripherally, on a distance of 2-5 cm on 4 patients with extensive BCC, it led to a complete remission of the tumor with excellent cosmetic and clinical results during a follow up period of 27 months. If for the patients with localized lesions the use of systemic PS is not justified because of the generalized photosensitizing, general photosensitizers (Porfimer Sodium or mTHPC) are recommended in the treatment of patients with multiple lesions. The results of a study with Porfimer Sodium on 1400 persons with superficial or nodular BCC showed a cure rate of 91%. PDT with mTHPC is efficient in multiple BCC, its advantage being the short duration of the treatment and the reduced radiation dose (Triesscheijn et al, 2006).

5.1.2 Squamous cell carcinoma

Because of the risk of metastatic disease, the use of PDT is restricted to in situ SCC. Bowen's disease is the pathology with the best response to PDT. There are numerous open, randomized trials assessing the effect of ALA-PDT in Bowen's disease. ALA combined with red light has significantly better results than the ones obtained with 5-fluorouracil or cryotherapy at 12 months follow-up with good or excellent cosmetic outcome (Morton et al,

2002). Comparing the effects of 20% ALA-PDT and fluorouracil therapy, a bicentric randomized trial showed a complete response in 88% of the lesions treated with ALA-PDT and only 67% after treatment with fluorouracil (Salim et al, 2003). However, literature data regarding the effect of PDT in Bowen's disease are controversial, some researchers reporting positive results in 90-100%, while others only in 50% of the cases (Fijan et al, 1995).

5.1.3 Other cutaneous cancers

PDT was also studied on the Kaposi sarcoma and the efficiency is similar to that of other treatments (Kalka et al, 2000). Various clinical studies have shown encouraging results for PDT in cutaneous lympho-proliferations. Still, there are no controlled trials on this matter, only isolated cases, treated topically, which can't, unfortunately, prevent the formation of new lesions. PDT is more of an additional therapy in treatment resistant T cell lymphomas (Kalka et al, 2000; Nayak, 2005). Because of the high melanin content of the melanoma, light penetration is greatly reduced. Less pigmented lesions respond better to treatment but still PDT is not a therapeutic option in melanoma. PDT is used with a palliative purpose in cutaneous metastases from breast cancer with the exception of inflammatory carcinoma which responds poorly to treatment and leads to important complications, like pain and cutaneous necrosis (Kalka et al, 2000; Nayak, 2005). PDT with systemically administered PS was used in the extramammary Paget disease resistant to conventional therapy, in severe lip displasia, and in actinic cheilitis.

5.1.4 Premalignant lesions

When treating AK one has many therapeutic options, such as: 5-Fluorouracil, podophyllin, Imiquimod, PDT, cryotherapy, etc. Numerous studies have demonstrated the effectiveness of PDT in treating AK. The response rate found in studies evaluating especially the AK of the face and skull was between 71-100% after one session of PDT. The best results were obtained when using red light (635nm) or blue light (417nm), the green light does not ensure a deep enough penetration in the lesion. An European multicentric, randomized, prospective study which compared MAL-PDT (one session) with cryosurgery in 193 patients having 699 lesions found no difference at 3 months after the treatment between the efficiency of the two methods (PDT 69% versus cryosurgery 75%) and found a small difference regarding the cosmetic results (96% versus 81%) (Szeimies et al, 2002). A study that took place in Australia involving 204 patients with AK compared the effects of 2 sessions of PDT with one session of cryotherapy and to placebo. At 3 months complete remission was reported in 91% of the patients that underwent PDT, 68% with cryotherapy, and 30% of patients who received placebo. The cosmetic results were excellent in 81% of the patients treated with PDT and in only 51% of the cases that received cryotherapy (Freeman et al, 2003). In the US, a randomized, double-blind, placebo controlled, multicentric study on 80 patients with AK treated with MAL-PDT in two sessions with red light (75 J/cm²) found a complete remission in 89% of the cases compared to placebo (38%), with excellent cosmetic results in 90% of the cases. Also a randomized, placebo controlled, double-blind study on 17 patients with 129 lesions of AK treated with MAL-PDT showed after 16 weeks a complete response in 13 patients of the total of 17 that entered the study. This method is considered safe and efficient in patients who received a transplant and have a high risk for the transformation of AK into SCC (Dragieva et al, 2004). The incubation period of ALA does not influence the therapeutic effect. A proof in this respect are the results of a study on 18 patients, each with at least 4

lesions of AK, that were followed for 4 months and who had a reduction of 90% of the lesions without significant differences regarding the incubation period of ALA. Often, hyperkeratosis can be a reason for therapeutic insuccess. In this case removing hyperkeratosis through gentle abrasion or non-bleeding curettage is necessary before the topical application of PS (Yang et al, 2003).

To obtain an optimal result the incubation period must be about 14-18 hours. Usually ALA products are applied topically on the lesion using protection for the neighboring tissue, 4 to 6 hours before irradiation. When using MAL unguent a 3 hour incubation period is sufficient because this PS has a high selectivity and is preferentially retained by the tumor (Foley, 2003). A burning sensation or pain may appear, especially during the irradiation or a few hours after (Morton et al, 2002). There are studies that show that PDT with MAL is less painful than PDT with ALA (Wiegell et al, 2003). If the treatment is applied on large surfaces, analgesic treatment may be used or the area can be ventilated using cold air (Szeimies et al, 2005). The use of topical unguents with lidocaine and prilocaine is not recommended because they can interact with ALA and inactivate ALA/MAL due to the local increase of the pH. A small discomfort may appear in the treated area because of erythema, edema or even due to dry necrosis, these symptoms disappear after 10-21 days when a complete re-epithelization is seen (Morton & Burden, 2001; Morton et al, 2002).

5.1.5 Conclusions

PDT is one of the most important advances in skin cancer therapy. This technique is valuable especially when size, site or number of lesions limit the efficacy of conventional therapies. The mechanisms involved in obtaining the effects of PDT are complex and intricate, and the results of the therapy depend on a series of parameters that can be appropriately modulated. Further studies must be carried out in order to improve the efficiency of this modality of treatment.

6. References

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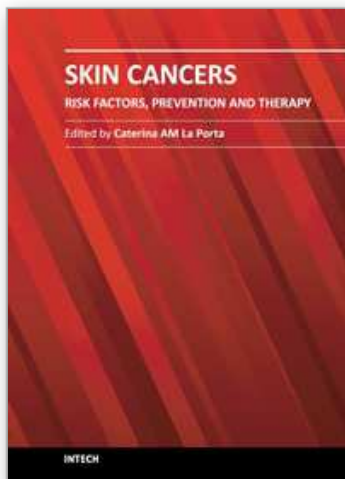
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Skin cancers are the fastest growing type of cancer in the United States and represent the most commonly diagnosed malignancy, surpassing lung, breast, colorectal and prostate cancer. In Europe, the British Isles have been the highest rates of skin cancer in children and adolescents. The overall idea of this book is to provide the reader with up to date information on the possible tools to use for prevention, diagnosis and treatment of skin cancer. Three main issues are discussed: risk factors, new diagnostic tools for prevention and strategies for prevention and treatment of skin cancer using natural compounds or nano-particle drug delivery and photodynamic therapy.

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