Proceeding of The 1st University of Muhammadiyah Purwokerto – Pharmacy International Conference
5th-6th June 2015, Horison Hotel Purwokerto
ISBN 978-602-73538-0-0

Photodynamic Therapy for Cancer Treatment

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ABSTRACT

The use of photosensitizers combined with visible non-toxic light has been known for more than hundreds of years, but now the application is just beginning to be widely used. This method is known as photodynamic therapy (PDT). PDT was originally developed to treat cancer. The procedure requires photosensitizing drug exposure to cells followed by irradiation of visible light at the appropriate wavelength, usually in a red or near IR that compatible with the absorption spectrum of the drug. After the photon has been absorbed, the photosensitizer will be excited and undergoing a transition which will eventually appear in its excited triplet state. The triplet state will transfer its energy to the ground state oxygen and produce molecular oxygen singlet which then causes damage to the cancer cell. Various photosensitizer compounds have been developed since the discovery of the first generation photosensitizer, hematoporphyrin and its derivatives, which shows some limitations. The ideal photosensitizer should be a pure, stable compound, not toxic in the absence of light, can be localized in cancer cells with high selectivity, and can be eliminated from the body quickly to avoid generalized skin photosensitization. For these reasons, second generation (and then third generation) of photosensitizers have been developed.

Key words: cancer, photosensitizers, photodynamic therapy (PDT).

INTRODUCTION

Photodynamic therapy in sort term is treatment, involves administration of photosensitizer (PS) followed by activation of the PS by light of a specific wavelength triggering in a sequence of photochemical and photobiological processes that effect irreversible damage of cancer cells. In the existence of oxygen, a chains of events lead to direct cancer cell death, damage to the microvasculature, and stimulation of a local inflammatory response (Agostinis et al., 2011).

PDT consists of 3 important components (photosensitizer, oxygen, and light at the appropriate wavelength). None of these components are toxic, but when they take action together, they initiate a photochemical reaction which ultimately produces highly reaction species termed singlet oxygen ($^1O_2$). One important thing about this process is that the light used is not of high energy (unlike X-ray radiation), so this light is harmless for life (Bonnett, 2002).

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Antitumor effects of PDT arise from 3 inter-related appliances: direct cytotoxic effects on tumor cells, destruction to the tumor vasculature, and induction of a inflammatory reaction that can initiate the development of systemic immunity. The relative contribution of the mechanism depends on the type and dose of PS used, the time between PS administration and exposure to light, the dose of light and its fluence rate, the concentration of oxygen in tumor cells, and other factors that are still unknown. Therefore, determining the optimal conditions for the use of PDT requires a combination of various scientific disciplines. This paper reviews the current conditions of PDT application, the biological and physicochemical aspects that are important in PDT, the mechanism of action, and the latest development of the PDT method in treating cancer.

HISTORICAL ASPECTS

Treatments that use light and compounds activated by light have existed since the ancient times, and have been used to treat various diseases. The use of chemical compounds as PS which replaces natural chromophore, was first developed by Raab (1900) and Jesionek - Von Tappeiner (1903). Raab adding dyes to petri dishes of paramecia caused in unexplained death for the duration of daylight experiments, but not for the duration of evening experiments. Instead of ignoring these findings, Raab systematically verified the association between light activation of these dyes and therapeutic effect. Jesionek and Tappenier reported the results of their experiments in which tumours is treated with eosin topically and then subjected to visible light.

In 1961 Lipsin and his research group had succeeded in making a hematoporphyrin derivative (HPD). This compound can be localized in tumor cells. Tissue uptake of HPD is characterized by the red fluorescence. The mixture of porphyrin in hematoporphyrin derivative is obtained by adding a mixture of glacial acetic and sulfuric acids to the commercial hematoporphyrin. Furthermore, the product is neutralized and washed to produce a product in the form of brown powder. In 1975, Kelley and Snell used HPD in a bladder cancer patient by PDT methods. Then, in 1978 Dougherty's group at the Roswell Park Cancer Institute reported the results of PDT in 25 patients with recurrent skin cancer.

OVERVIEW OF A PDT PROCEDURE

Photofrin (PF) is given by intravenous injection 24-48 hours before irradiation. Because of skin photosensitization, patients must avoid direct sunlight for around 6 weeks. The fluorescence of the drug varies from barely visible to bright depending on the location of the tumor. The PDT lamp used is an argon-ion pumped tunable dye laser at 630 nm or another sources. Superficial tumors are irradiated using external optical fibers with "microlens" to provide uniform radiation. Deep lesions can be treated by inserting the tip of the optical fiber in the tumor mass. Endoscopic techniques are used for PDT interstitial tumors, for example, cylindrical diffuser in the esophagus and ball diffuser in the bladder. PDT treatment time ranges from a few minutes to more than one hour, depending on the size of the tumor, drug dosage, laser strength, and light delivery mode. Bleaching of tumor tissue and diffuse

ISBN 978-602-73538-0-0

bleeding may be evident during or immediately after irradiation of light. Tissue necrosis and eschar formation progress over a period of 2-3 weeks, followed by healing after about 6-8 weeks. The necrosed area becomes almost natural in time, perhaps because PDT does not destruction collagen (Grossweiner, 1994).

PHOTOCHEMISTRY AND PHOTOPHYSICS (Agostinis et al., 2011)

Most PS in their ground state (ie, singlet) have 2 electrons with opposite spins located in the molecular orbitals which are most energetically beneficial. Absorption of light causes the transfer of one electron to a higher energy orbital (Fig 1).

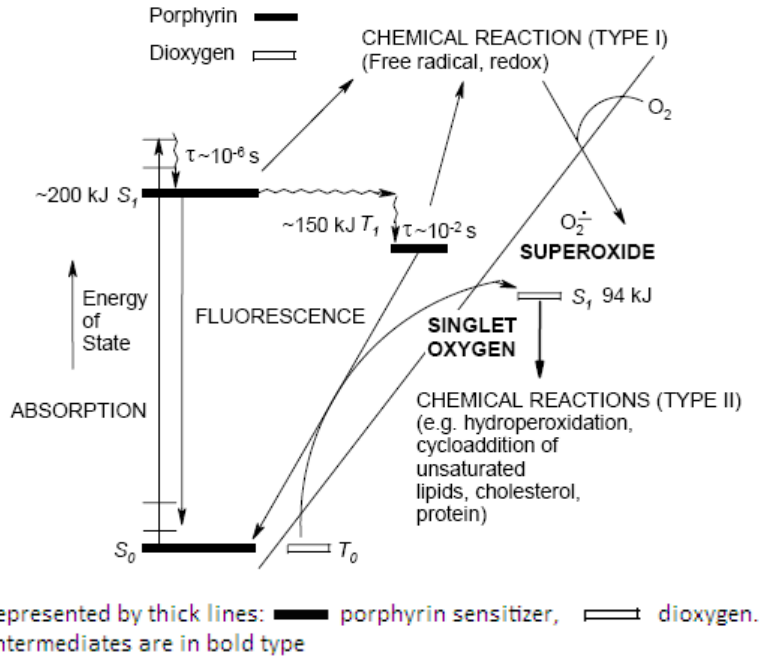

Fig 1. Generation of excited photosensitizer porphyrin states and reactive dioxygene species (Kral et al., 2006).

The excited PS is very unstable and radiates this excess energy as fluorescence and/or heat. Instead, an excited photosensitizer may undergo an intersystem crossing to form a more stable triplet state with inverted spin of one electron. The PS in triplet state can also decay radiationlessly to the ground state or transfer its energy to molecular oxygen (O_2), which is distinctive in being a triplet in its ground state. This step induce the formation of $^1O_2$, and the reaction is denoted to as a Type II process (Foote, 1968). A Type I can also occur when the PS reacts directly with an organic molecule in a cellular microenvironment, acquiring a hydrogen atom or electron to form radicals. Subsequent autoxidation of the reduced PS yields a superoxide anion radical ($O_2^{\cdot-}$). Dismutation or one electron reduction of $O_2^{\cdot-}$ provides hydrogen peroxide (H_2O_2), which in order can endure one-electron reduction to

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a powerful oxidant hydroxyl radical (HO•). Reactive oxygen species (ROS) generation via Type II is mechanistically much simpler than via Type I, and most photosensitizers are believed to operate through a Type II mechanism rather than Type I.

PHOTOSENSITIZERS

PS are chemicals that are accomplished of absorbing light of specific wavelength (chromophores) and converting it into valuable energy. In the case of PDT, this would implicate the production of lethal cytotoxic substances Based on this definition, there are hundreds of natural and synthetic dyes ranging from plant extracts to synthetic macrocycles can function as PS for PDT. However, a good photosensitizer is expected to meet the following minimum criteria (Maiya, 2000). It should:

1. Have a strong absorbance with a high extinction coefficient (ε) in the longer wavelengths area (600-850 nm) where the light penetration in the tissue will be maximum and still have energy to produce $^1O_2$.
2. Have good photochemical reactivity, with high quantum yields of triplet state (Φ_T) and long triplet state lifetimes and can produce $^1O_2$ and other reactive oxygen species effectively.
3. Have minimal dark toxicity and only be cytotoxic in the presence of light.
4. Be specially retained by the target tissue.
5. Be fast eliminated from the body, therefore inducing a low systemic toxicity.
6. Be chemically pure and of known composition.

Among these six major criteria, the first two are the most essential ones and the reasons behind these strict optical and photophysical criteria are discussed below.

In order to criticize clinically available photosensitizers, one must have an ideal kind of comparison. However, the ideal photosensitizer would vary from doctor to purist. The criteria that follow are clinically relevant (Allison et al., 2004).

1. Toxicity, Ps is not a toxic chemical. Furthermore, photosensitizer metabolism should not create new toxic byproducts.
2. Mutagenicity/carcinogenicity, the PS should not cure one disease just to make another.
3. Selectivity, a PS that selectively accumulates in the tissues is very useful. Intracellular targets, such as the mitochondrial membrane, will cause the death of cancer cells programmed by apoptosis. Cell membrane or extra cellular death through damage to blood vessels will cause necrosis. Clearly, the target of PDT can be important and have clinical concerns. One may be capable to exploit this for producing PDT vaccines via encouraging system reaction or highly selective apoptotic reaction (Konan et al., 2002). Moreover, one could conjugate the photosensitizer, for example, to carriers, radioactive source, monoclonal antibodies, etc. to improve specificity and destructive ability.
4. Elimination, elimination of the PS from the body should be of clinical helpfulness.
5. Activation, consistent activation by an appropriate wavelength of light is required to avoid accidental treatment.
6. Sunlight protections, as all PS go to skin, some degree of sunlight protections are desirable. Preferably, this would be measured in hours or days and not weeks or months.
7. Administration, adaptable by topical, swallowing, inhalation or IV, depending on the clinical condition. However, minimal administrative toxicity (i.e. hypotension, allergic reaction) and ease of administration are valuable features.

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8. Indications, would it be better to have very specific drugs for certain medical indications or one PS that works on most diseases?
9. Reliability, indeed the best theoretical PS must get where you need it and activate it when you need it, every time, or almost useless.
10. Pain-free therapy, because PDT is performed in outpatient and usually does not need sedation, a PS which induces pain during and after therapy will not allow outpatient.
11. Outpatient therapy. PDT is also cost effective. Because the cost of therapy plays a greater role in insurance decisions, keeping PDT less costly than other therapies is important. Patients also prefer outpatient care over hospitalization.
12. Availability, the PS should be commercially available and can be reconstituted by a local pharmacy instead of sub specialty labs.
13. Cost, very expensive drugs will prevent their widespread use.
14. Safety
15. Biochemistry, water-soluble PS easily passes through the body. With chemical modification non-soluble PS can be synthesized with right carriers to allow for clinical use.
16. Wavelength, longer wavelengths of PS activation permit for deeper tissue penetration. Activation at 400 nm permit a millimeter light depth penetration; 630 nm gives about 10 mm depth penetration.
17. Integrative capacity, an optimal photosensitizer will be capable to be used in conjunction with other forms of modality such as surgery, radiation, and chemotherapy.
18. Forgiving, with restricted dosimetry available highly active PS can easily permit treatment overdosage. Less active PS may be more forgiving of excess radiation.

In the visible wavelength region, hemoglobin and melanin are the main endogenous chromophores of the skin. Hemoglobin has strong absorption bands near 418, 542 and 577 nm and melanin absorbs over a broad range with a maximum in the 300-500 nm region, but decreasing steadily in the visible range. Hemoglobin has a strong absorption band in areas 418, 542 and 577 nm and melanin absorbs a maximum of 300-500 nm, but its absorption continues to decrease in the visible light region. Therefore it is very important to use the illumination area at a wavelength of more than 600 nm. At wavelengths longer than 1000 nm, light absorption by water molecules serve significant. Therefore, there is a 600-1000 nm 'therapeutic window' that allow substantial light penetration into the tissue. Within this therapeutic window, longer wavelengths penetrate more deeply because of decreasing tissue absorbance and decreasing light scattering. However, for wavelengths greater than 850-900 nm, the photons may not have enough energy to play a part in photochemical reactions. The region between 600 and 1200 nm is often called the optical window of tissue. However, light up to only about 800 nm can generate $^1O_2$, because longer wavelengths have not enough energy to initiate a photodynamic reaction (Juzeniene et al., 2006). Therefore, the available wavelengths for PS are 600-850 nm (red light). In general, the use of PS with stronger absorption (large extinction coefficient) offers the probability to inject smaller drug doses (Maiya, 2000).

First-Generation PDT Drugs

In the 1970s hematoporphyrin (Hp) and HpD were the most commonly used PS. They were later reffered to the first generation PS. HpD is a mixture of porphyrins. It was presented that HpD contained at least seven

ISBN 978-602-73538-0-0

compounds, both hydrophilic and lipophilic. These compounds have different intracellular and tumour localization characteristic, and photosensitivities (Juzeniene and Moan, 2007). First-generation PDT sensitizers for example Photofrin®, shown prolonged patient photosensitivity (poor clearance) and lacked long wavelength absorption (MacDonald and Dougherty, 2001): main aspects contributing to the limitation of these PS in PDT (Josefsen and Boyle, 2008).

Second-Generation PDT Drugs

The synthesis of improved PS (second-generation) is done by modifying the tetrapyrrolic (porphyrin) structure, such as porphycene (ATMPn), chlorin (Temoporfin®), and benzoporphyrin (Visudyne®), which have a more intense long wavelength absorption (MacDonald and Dougherty, 2001; Brown et al*.,* 2004). Metallated derivatives of porphyrin have also been synthesized include [Al, $AlPcS_4$; Si, SiNC (Nc—naphthalocyanine)]; and Sn, $SnEt_2$ (Ali and van Lier, 1999), even though there is no consistent association between metallation and increased photodynamic activity (Josefsen and Boyle, 2008). Other PS including merocyanine 540 (MC 540), chlorin e6, sulfonated tetraphenylporphines, meso-tetra(hydroxyphenyl)chlorin (m-THPC), meso-tetra(3-hydroxyphenyl)porphine (m-THPP), hypericin, meso-tetra-(*N*-methyl-4-pyridyl)porphine ($TMPyPH_2$), methylene blue derivative (MBD) were calculated their physicochemical characteristic, intracellular/tissue uptake, localization, photodegradation and photodynamic activity both in vitro and in vivo by Moan Group Research (Juzeniene and Moan, 2007). In 2001, PDT using m-THPC (Foscan, Biolitec, the Netherlands) was accepted in the European Union, Iceland and Norway as a local therapy for the palliative treatment of patients with neck and head cancer.

Third-Generation PDT Drugs

At present, targeted strategies are carried out to increase PS affinity in tumor tissue (Hudson *et al.,* 2005). In 1997, Dummin *et al.* had reported selectivity of PS in subcellular compartments, including mitochondria. These targeting methods have led to third-generation PS (Josefsen and Boyle, 2008).

The report by Leung *et al.* (2008) show the need for increased selectivity of PS for tumour tissue over healthy tissue—without this specificity PDT has restricted clinical uses. The problem can be overcome by increasing photosensitizer affinity for tumor tissue by targeting specific photosensitizers against tumor/cell sites. Conjugating the targeting components, such as antibodies (directed against the tumour antigens), towards the photosensitizer permits the PS to localize, accumulate and bind selectively at the affected area (Konan et al*.,* 2002; Hudson et al., 2005; Staneloudi et al., 2007). The PS bioconjugate is at that point capable to (specifically) photodynamically inactivate in tumour cells expressing the tumour-associated antigen, reducing healthy cell localization and related damage. Other receptor-positive locates on the tumour surface, such as LDLs (low-density lipoproteins) and folate receptors can be used of by conjugating the PS to LDL or folate molecules (Konan et al., 2002): folate and LDL

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receptors are overexpressed on tumour cell surfaces. Some photosensitizers that have shown activity *in vivo,* have low solubility in aqueous media, this will reduce intravenous delivery into the bloodstream and affect their ability in physiological media and the clinic (Josefsen and Boyle, 2008).

Investigations into third-generation PS bearing targeting moieties (Konan et al., 2002; Hudson et al., 2005; Staneloudi et al., 2007) has proven that PS directed against tumour tissue has minimal accumulation in normal tissue, whereas a high tumour binding specificity is detected. Moreover, the previous study shown that PS conjugated to a single-chain monoclonal antibody (scFv) fragment is also more effectively eliminated from the circulation than PS–monoclonal antibody conjugate alone (Staneloudi et al., 2007).

The chemical structure of a PS plays a main role in the success of the drug as a PDT agent. PS needs to be soluble in physiological media: the degree of PS amphiphilicity and hydrophilicity directly affects its route of administration and the pharmacokinetic/biodistribution profile (Castano et al., 2005; MacDonald and Dougherty, 2001). PS that has certain structural characteristics has been reported to to be selectively localized in tumor tissue. Even though the mechanism of localization is not well understood, the more hydrophobic PS shows the ratio of localization of the tumor to normal tissue 7: 1 and 8: 1, whereas the more hydrophilic PS shows a ratio of 2: 1 (Ruck and Steiner, 1998).

PS with anionic substituent, such as sulfonate or carboxyl group, has been observed to localize preferentially in the cytoplasm and relocate to the nucleus upon illumination (Patito et al., 2001), whereas lipophilic PS function with cationic group is believed to (preferentially) across the mitochondrial membrane and accumulate in mitochondria (Dummin et al., 1997) - the subcellular organelles are widely demonstrated to be a key component in the preferred (apoptosis) cell death pathway. Exactly the physicochemical/structural characteristics and mechanisms behind this specific distribution and localization and how to maximize tumor tissue selectivity over normal tissue accumulation is a issue that is still under examination.

LIGHT SOURCES (Agostinis et al., 2011)

Penetration of blue light through tissue is less efficient than red and infrared radiations (Fig. 2). The choice of light source is therefore should be based on PS absorption (fluorescence and absorption spectra), disease (size of lesions, location, accessibility, and tissue properties), size, and cost. The clinical efficiency of PDT is dependent on complex dosimetry including total light dosage, light irradiation time, and light delivery method (single vs fractionated or even metronomic). The fluence rate also impact PDT response (Henderson et al., 2006).

Lasers and incandescent light sources have been used for PDT and indication similar efficacies (Brancaleon and Moseley, 2001). Unlike the large and inefficient pumped dye lasers, diode laser is small and cost-effective, is simple to install, and has automated dosimetry and calibration features and a longer lifetime. Such laser is now being

ISBN 978-602-73538-0-0

specially designed for PDT. Light-emitting diode (LEDs) is alternative light source with relatively narrow spectral bandwidth and high fluence rate (Juzeniene et al., 2004; Szeimies et al., 2005).

Lasers can be combined into fibers with diffuse tips to treat tumors in the bladder and digestive tract. Inflatable balloons, covered inside with strong scattering materials and formed to fit organs, are also commercially available (Beyer, 1996). It is feasible to infuse a light source in a solid organ deep in the body under the guidance of the image. The choice of the optimal combination of PS, light sources, and treatment parameters are very important for the success of PDT (Wilson and Patterson, 2008; Plaetzer et al., 2009).

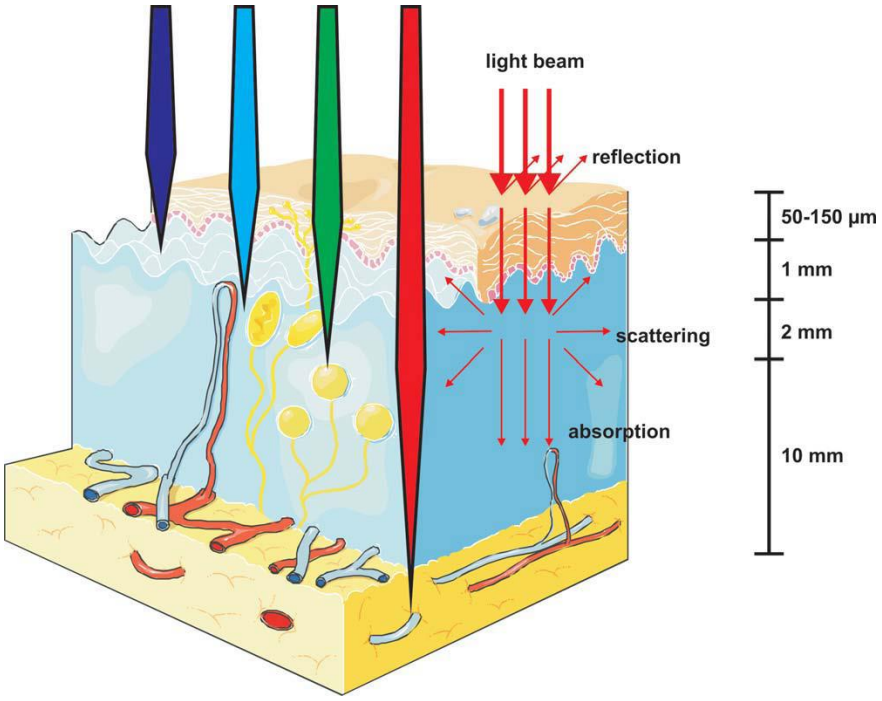

Fig 2. Light penetration through the tissues.

FLUORESCENCE AND PHOTODYNAMIC DIAGNOSIS (PDD)

Photodynamic diagnosis (PDD) involves fluorescence to observe PS localization in abnormal tissue. Thus, this technique is also called fluorescence diagnosis or fluorescence photodetection. Representing an additional optical recognition condition, PDD exposes neoplastic lesions that cannot be seen through conventional techniques. The added value depends on the accumulation of fluorochrome selectively and on how well disturbing optical tissue inhomogeneities can be considered or eliminated (Jocham et al., 2008).

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Fluorescence is produced by the interaction of light (photons) with the outer electron of molecule. Molecule that is electronically excited by absorption of a photon has several options to coming back to their ground state. One option is to emit a secondary photon. Fluorochromes absorb light with a high energy per photon and re-emit light with a lower energy per photon, generating a shift in colour between excitation and fluorescence light (Jocham et al., 2008).

The use of exogenous drugs for fluorescence diagnosis depending on a positive fluorescence mark of tumor tissue. Most of the fluorochromes that already were used in the clinic environment are photosensitisers. Besides showing fluorescence, photosensitisers also employ a phototoxic action, which is used for photodynamic therapy (PDT) (Jocham et al., 2008).

Moreover, fluorescent spectra can distinguish benign and malignant tumor and avoid therapy to normal tissues (Gillenwater et al., 1998). In conclusion, we can imagine that the difference in fluorescence prior, during, and after therapy might be used to monitor the prospective or failure of treatment (Braichotte et al., 1996). However, the sum of fluorescence and PDT is unity, so the more powerful a fluorescent indicator, the less active the PDT drug and vice versa (Allison et al., 2004).

MECHANISMS OF PDT-MEDIATED CYTOTOXICITY (Agostinis et al., 2011)

The lifetime of singlet oxygen is very short (about 10-320 nanoseconds), restrictive its diffusion to only about 10 nm to 55 nm in cells (Dysart and Patterson, 2005). Therefore, photodynamic damage will take place very close to the intracellular location of the PS (Moan et al., 1989). PDT can induce the 3 main cell death ways: apoptotic, necrotic, and autophagy-associated cell death (Fig. 3). Apoptosis is a commonly major cell death type in cells associating to PDT. Mitochondria outer membrane permeabilization (MOMP) after photodynamic damage is managed by Bcl-2 family members and considered mostly p53-independent (Buytaert et al., 2007). With mitochondria-associated PS, photodamage to membrane-bound Bcl-2 (Kessel and Castelli, 2001; Xue et al., 2001; Usuda et al., 2003) can be a permissive signal for MOMP and subsequent releases of caspase activators such as cytochrome c and Smac/DIABLO,
or other proapoptotic molecules, including apoptosis-inducing factor (AIF) (Buytaert et al., 2007).

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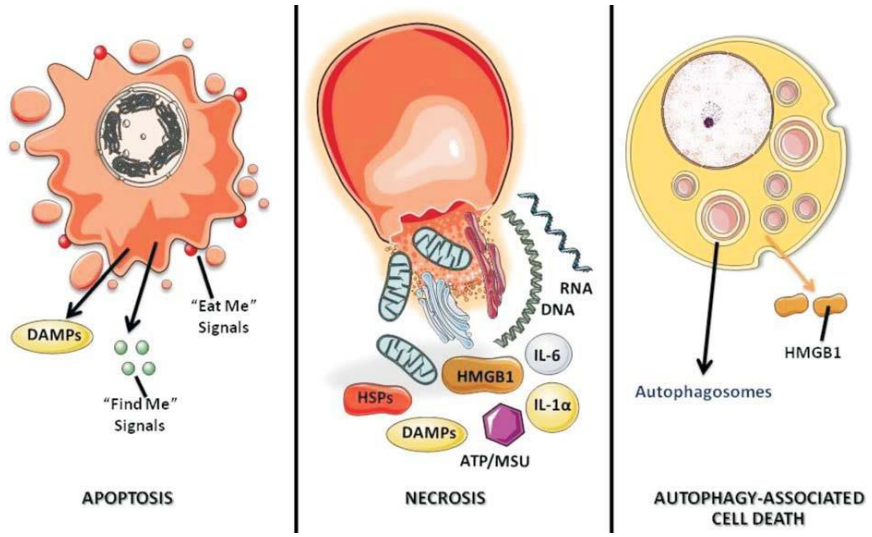

Fig 3. Three main cell death morphotypes and their immunological characteristic.

Photodamage of tumor cells can also initiate stimulation of macroautophagy (Reiners and Agostinis, 2010; Buytaert et al., 2007). This is a lysosomal pathway for the degradation and recycling of intracellular organelles and proteins. This route can have both a prodeath and a cytoprotective part after cancer chemotherapies, containing those involving ROS as main damaging type. Current results define autophagy as a mechanism to preserve cell viability after photodynamic damage (Reiners and Agostinis, 2010). PS that photodamage the lysosomal part can compromise completion of the autophagic route, affecting incomplete allowance of the autophagic cargo. Accumulation of ROS-injured cytoplasmic parts may then potentiate phototoxicity in apoptosis-competent tumor cells (Reiners and Agostinis, 2010). A better understanding of the relationship between autophagy, necrosis, and apoptosis and how these processes cause enhanced tumor response will be a necessary to develop better therapeutic approaches in PDT.

MECHANISM OF THE TUMOR LOCALISING EFFECT IN PDT (Kral et al., 2006)

a. Cancer cells, could have an increased necessity for cholesterol for membrane biosynthesis. They can hence upregulate the expression of the low-density lipoprotein (LDL) receptor. It is recognized that lipoprotein is main carrier of lipophilic porphyrins in the bloodstream and might therefore be a means of admission of these compounds into cancer cells.
b. Tumours frequently have increased amounts of lipid bodies and mostly neutral lipid droplets, in addition their cell membranes may perhaps be more hydrophobic than those of normal cells. Both phenomena may describe the accumulation of hydrophobic PS.
c. A decreased intratumoral pH can affect the ionization of porphyrin compounds with weakly acidic pK values, consequently retaining them within tumours.
d. Tumour cells can have enhanced abilities for phagocytosis or pinocytosis of porphyrin aggregates.

ISBN 978-602-73538-0-0

e. A combination of "leaky" tumour vasculature and reduced lymphatic drainage may stimulate the build-up of porphyrins (whether as proteincomplexes or aggregates) in the interstitial space.
f. Tumour-associated macrophages (TAM) can be mainly responsible for the concentration in tumours. It has detected that TAM may have up to nine times the porphyrin levels existing in tumour cells. Several experimental tumours may comprise up to 80% TAM.

PDT convinces a highly complex series of alteration in cells. The sequence of actions in PDT are displayed in following figure. It can be seen that the establishment of a complete protocol requires a broader study of biochemical and photochemical phenomena (Fig 4). It is tends to affect several cell targets, where the cell membrane and mitochondria are the main targets, followed by lysosomes, endoplasmic reticulum, DNA and microtubules. After exposure, cells experience a fast increase in calcium concentration accompanied by other electrolyte changes as membrane destruction takes place. Sublethal destruction may result in apoptosis described by a drop in mitochondrial potential, simultaneous with a drop in ATP level and a decrease in cell respiration, translocation of phosphatidylserine of the plasma membrane, DNA fragmentation, appearance of apoptotic bodies and finally loss of plasma membrane integrity. The association of constituents of signalling network such as cell surface death receptor Fas, tumor necrosis factor (TNF) and TNF-related apoptosis-inducing ligand TRAIL other than downstream molecules such as caspases and Bcl-2 family members have been confirmed in various PDT-induced models of cell death. Recently, protein phosphorylation as an main regulator of the apoptotic process has been underlined. Apoptotic signalling cascade in photosensitized human epidermal carcinoma cells was mediated by two-stage activation of the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK).

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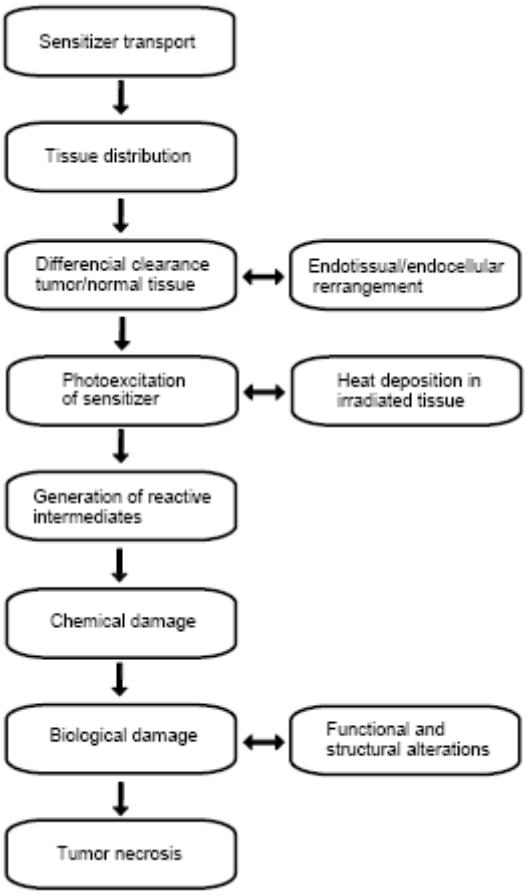

Fig 4. Sequence of actions in PDT.

CONCLUSIONS

It has been proven that PDT has the prospective to become a main method for cancer treatment. The photosensitizer (as drug) will be capable to monitor for a medical situation over fluorescence, optically biopsy the lesion for diagnosis, and treat the injury by PDT. The interdisciplinary exclusivity of PDT enthuses researcher in physics, chemistry, biology, and medicine to improve the treatment.

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