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REVIEW

PHOTODYNAMIC THERAPY (PDT): A PHOTOCHEMICAL CONCEPT WITH MEDICAL APPLICATIONS

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The development of new drugs (known as photosensitizers) for photodynamic therapy (PDT) requires the comprehension the way of these drugs works in biological medium. PDT is based on a systemic or topical administration of a tumor-localizing photosensitizers (PS) followed by illumination with visible light of appropriate wavelength. The resulting photodynamic reactions lead to the production of singlet oxygen ($^1O_2$) and many other reactive oxygen species (ROS's) that induced the tumor necrosis or apoptosis. Most of the PS are macrocycles (*i.e.* Porphyrins, Phthalocyanines, chlorines, etc.), are difficult to administrate and biodistribute *in vivo*. The main goal of this paper is the developing and elucidating the mechanisms of photo destruction of neoplastic tissue using new PS, evaluating the photophysical, photochemical and photobiological properties of some PS.

INTRODUCTION

The utility of light as a therapeutic agent can be traced back over thousands of years when it was used in Ancient Egypt, India and China to treat a variety of skin diseases like psoriasis, vitiligo, rickets, cancer and psychosis. The isolation of porphyrins and their inherent tumor localizing properties coupled with its ability to generate reactive singlet oxygen when activated by light of particular wavelength which in turn results in cytotoxicity led to the emergence of a new modality namely, photodynamic therapy (PDT) as a therapeutic tool.[1] Porphyrins represent one class of molecules under intense investigation due to their photosensitizing ability for PDT application. These chromophores show strong absorptions in the red region (650-800 nm) compared to that of normal 18 π porphyrins. The strong absorption of light by a water soluble nontoxic photosensitizing molecule in the therapeutic window resulting in maximum penetration of light into the tissues coupled with high singlet oxygen production will conceptualize an ideal photosensitizer. This review highlights various porphyrin sensitizers reported till date and their photosensitizing ability both *in vitro* and *in vivo* studies.

Photodynamic therapy (PDT) is a form of photochemotherapy requiring the simultaneous presence of a photosensitiser, activating light of the proper wavelength and molecular oxygen in order to produce a localised therapeutic effect thought to be due to high-energy singlet oxygen generation. Neither drug nor light alone are effective as therapeutic agents and thus PDT treatment methods should be looked upon as true, necessary, drug and device combinations ('systems'). Selectivity of treatment is imparted by a combination of factors, including accumulation of photosensitiser by the target lesion and targeted application of activating light. The most common systemic side effect of systemically administered photosensitisers is cutaneous photosensitivity of varying periods of time. Local toxicities depend on the area of treatment. Sources of light which have been used in PDT include lasers, arc lamps, light-emitting diodes and fluorescent lamps. PDT has been used for a wide variety of clinical applications.[2]

The first investigation of photodynamic destruction of malignant tumors was described in 1966 R. L. Lipson with co-workers harnessed the potential effect of selective destruction of tumors containing HpD. The destruction was performed due to the HpD photodynamic properties. The investigation was performed in a female patient with an extended recurrent ulcerated cancer of the mammary gland. Hematoporphyrin derivative was introduced repeatedly to the patient. The tumor was exposed to xenon-lamp light that had passed through a filter (the radiation spectrum is

unknown). The results obtained were reported at the Ninth International Cancer Congress in Tokyo in 1966 and explained the final tumor disappearance.[3-6]

In 1978, T. J. Dougherty with co-workers applied HpD PDT in the treatment of cancer patients[7-9]. They treated 113 cutaneous or subcutaneous malignant tumors. The researchers observed a total or partial resolution of 111 tumors. Extended or pigmented tumors required large HpD doses. To avoid damaging of the normal skin, they needed either to decrease light doses or to increase the time interval between photosensitizer injections and light exposures. The researchers believed that the laser should be a good alternative to arc lamps. With this end in view, they employed a tunable dye laser with argon pumping. Laser radiation was delivered *via* light-guiding fibers.

Later (in 1980), the red laser irradiated small foci of endobronchial carcinoma. The radiation was delivered via flexible light-guiding fibers made of quartz. The fibers were inserted into the tool channel of a flexible bronchoscope. [10-15] A pronounced therapeutic effect was observed in 91 percent of the patients, using Photohem which is a complete analog of Photofrin II in Russia. By now, more than 1,500 patients have undergone PDT with Photohem. Of these, 62 percent showed a total tumor resolution, whereas 29 percent showed a partial tumor resolution (the tumor halved in size in them). Early-diagnosed tumors disappeared completely in 92 percent of the patients.

The last several years have seen a considerable progress in antibacterial therapy. However, the problem of infectious diseases ranks high in many medical fields. At present, there are many antibiotic-resistant germs, with Escherichia coli, Staphylococcus aureus, and Streptococci being the most aggressive and resistant bacteria.[16] In the case of a sepsis, staphylococci, fungi, and enterococci are the most resistant germs. The resistance of germs to antibiotics and the need for systemic treatment cause many secondary problems (such as nephro-, hepato-, and neurotoxicities). Among such problems is systemic toxicity of antibacterial compounds.[17]

MECHANISM OF THE PDT PROCESS

Photosensitisers have a stable electronic configuration which is in a singlet state in their lowest or ground state energy level. Following absorption of a photon of light of specific wavelength a molecule is promoted to an excited state, which is also a singlet state and which is short lived. The photosensitiser returns to the ground state by emitting a photon (fluorescence) or by internal conversion with energy loss as heat. It is also possible that the molecule may convert to the triplet state via intersystem crossing which involves a change in the spin of an electron. The triplet state photosensitiser has lower energy than the singlet state, but has a longer lifetime (typically > 500 ns for photosensitisers) and this increases the probability of energy transfer to other molecules. There are two mechanisms by which the triplet state photosensitiser can react with biomolecules; these are known as the Type I and Type II reactions, the first involves electron/hydrogen transfer directly from the photosensitiser, producing ions, or electron/hydrogen abstraction from a substrate molecule to form free radicals, and the second producing the electronically excited and highly reactive state of oxygen known as singlet oxygen.[18]

Light can induce chemical, physical and biological effects within macromolecules and tissues. Depending on the power density, light and laser light in particular cause different effects in biological tissues like photochemical reactions, coagulation, photo- and thermal ablation, plasma formation and photodisruption.[19] The photochemical interaction occurs at very small power densities (0.01 - 50 W/cm^2) and plays significant role during the PDT. To perform this therapy, spectrally adapted chromophores are injected into the body. Irradiation with a suitable wavelength may trigger selective photochemical reactions, resulting in desirable biological transformations. A natural or synthetic chromophore compound capable of causing light-induced reactions in other non-absorbing molecules after resonant excitation is called photosensitiser (PS). The excited state of the PS decays through diverse channels resulting in intra-molecular transfer reactions and release of highly cytotoxic species.

Most photosensitisers are organic dyes. Their electronic states are characterized by singlet states (total electron spin momentum s = 0) and triplet states (s = 1). Each electronic state is subdivided in a band of vibrational states. The ground state of the photosensitiser is a singlet state. The excited states may be singlet or triplet states. After resonant absorption of light, the PS goes into a singlet excited state $^1S^*$, in which the spins of the excited and unexcited electrons are still paired. This process occurs very rapidly (10^{-15}s). The direct

transition from a ground singlet state into an excited triplet state is forbidden. There are three potential decay channels from the excited singlet state: non-radiative and radiative decay to the singlet ground state, and intersystem crossing to a long-lived excited triplet state. The radiative singlet decay is called fluorescence; it is a highly probable process observed nanoseconds after the excitation. The first excited singlet state can participate in an electron transfer process with a biological substrate, resulting in the photobleaching of the photosensitizer and modification (destruction/ inactivation) of the substrate. Intersystem crossing (ISC) is another possible fate of the excited singlet state.[20] ISC requires a spin flip so that both electrons have parallel spins, giving a triplet excited state of the sensitizer, $^3S^*$**.** Triplet states are metastable because the spontaneous conversion to the singlet ground state requires another spin flip, which is a forbidden (low probability) transition. ISC is promoted by heavy atoms in the molecular structure or in the medium. The radiative decay of a triplet state leads to a weak light emission termed phosphorescence which is observed miliseconds or even seconds after the absorption. Dye triplet states are chemically reactive owing to their relatively long decay lifetimes and the presence of unpaired valence electrons. Two alternative reaction mechanisms with surrounding molecules for the decay of the triplet state are available. They are called type I and type II reactions: an electron transfer process (Type I reaction) and an energy transfer to ground-state molecular oxygen (Type II reaction).

The type I reactions lead to generation of free radicals when the PS in the triplet state reacts with a target molecule, other than oxygen. The PS may interact also with a triplet oxygen which leads to formation of hydrogen dioxide or superoxide anions. In a type II pathway, $^1O_2$ is generated by direct "downhill" energy transfer from the sensitiser triplet state to molecular oxygen $^3O_2$. The electron spins are flipped in the following manner:

$$\begin{array}{ccccccc} \uparrow\uparrow & & \uparrow\uparrow & & \uparrow\downarrow & & \uparrow\downarrow \\ {}^3S^* & + & {}^3O_2 & \rightarrow & {}^1S & + & {}^1O^* \end{array}$$

Molecular oxygen in its $^3\Sigma_g^-$ ground state has triplet multiplicity unlike most natural compounds. Chemical reactions forming singlet molecules from triplet and singlet reactants are forbidden by Wigner's spin selection rule. Thus, the triplet multiplicity is the actual reason, why most reactions of oxygen with organic substances do not proceed at room temperature but upon heating or in the presence of catalysts. This effect enables our life in an oxygen containing atmosphere. The lowest excited singlet state of O_2 lies by only 0.98 eV (94 kJ mol^{-1}) above the triplet ground state. This $^1\Delta_g$ state is commonly populated by energy transfer from the PS's triplet state whose energy relative to its $^1S_0$ ground state must exceed the excitation energy of $O_2(^1\Delta_g^+)$. The transition to the $^3\Sigma_g^-$ ground state is strictly forbidden for the isolated $O_2(^1\Delta_g)$ molecule. Nevertheless, in the condensed matter, the $O_2(^1\Delta_g)$ singlet oxygen is a metastable species due to collisions. Singlet oxygen has a lifetime of 0.6 μs in cellular environment, 4 μs in water and a little longer in lipids (50-100 μs) and cell membranes.[6] That means that the diffusion length of the singlet oxygen is of the order of the distance of the different organelles in cells and it cannot diffuse longer than a single cell length. Because of its singlet multiplicity no spin-forbiddeness exists for reactions of $O_2(^1\Delta_g)$. Therefore, and due to its excitation energy of 94 kJ mol^{-1}, singlet oxygen is chemically extraordinary reactive. Only little above the $^1\Delta_g$ excited singlet state lies the $^1\Sigma_g^+$ singlet state of the oxygen molecule with excitation energy 157 kJ mol^{-1}. The $^1\Sigma_g^+$ state is completely very fastly deactivated to the metastable $^1\Delta_g$ state in collisions with other molecules.

Reactive oxygen species: superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen are implicated as agents producing injury to cells and tissues, the last giving rise to adverse health effects.[21] The formed toxic oxygen species and free radicals are very reactive chemicals and can damage proteins, lipids (including phospholipids and cholesterol), nucleic acids and other cellular components and with certain α-aminoacids side chains in proteins (Trp, His, Met); these are all vital components of membranes (external and internal to the cell) and membrane damage in this way is a plausible macroscopic mechanism for cell damage and death.

Various optical devices can detect the fluorescence and the process has been under investigation for photosensitiser localization in tumor tissue.[22] A typical PDT session includes intravenous injection (I.V.) or topical application of a photosynthesizing agent. Some time is required for systemic porphyrins (I.V. injection) to be cleared from normal tissues and be preferentially retained by rapidly growing tissues, or for topical

porphyrins to be absorbed by the skin. After this period of time has been elapsed application of light to tumor site activates the photochemical reactions and produces an active form of oxygen that destroys the treated cancer cells.

Usually, the most used lasers are: He-Ne laser (λ= 632 nm, power 300 mW); Ar ion laser (λ=514.5 nm and fluence rate less 100 mW/cm^2); Ar ion laser (λ=488 nm) and the excimer laser (λ=248 nm pulsed with 50 mJ /pulse).[23] The main disadvantage of dye or Ar ion lasers is that the equipment contains expensive devices that require costly maintenance and staff training. To get around this limitation in PDT, it has been proposed to use diode lasers or a ´´light-emitting diode array´´(LED array). They are compact, simple to operate and can be plugged directly into a standard wall socket. They have enough power and operate at wavelengths compatible with currently used PSs.[24,25] They do suffer from the disadvantage that they are not tunable and may only be used at a fixed wavelength. However, for treatment with a particular drug for a given indication they are extremely convenient. Non-laser light sources are also finding increasing use in PDT, especially for dermatological applications and other types of treatment where the use of narrow fibres is not required as, for example, in treatment of the cervix. They are generally cheaper even than diode lasers.

The wavelength of radiation should correspond to an absorption peak in the PS's spectrum. For better penetration of radiation this peak should be far from the main absorption peaks in human tissues. In the UV and the visible part of the spectrum, absorption is due to proteins, melanin and hemoglobin in blood. The melanin is a basic pigment of the skin being one of the main epidermal chromophores. The linear absorption coefficient of the melanin increases smoothly from the visible to the UV part of the spectrum. The hemoglobin is characterized with absorption peaks at wavelengths 280 nm, 410 nm, 540 nm, 580 nm and about 600 nm. Above 1 μm absorption in tissues follows the absorption spectrum of the water molecule due to the high percentage of water in tissues (more than 60% in soft tissues). The most suitable for the PDT is the region between 600 and 1200 nm. Being free of absorption peaks, it is called a therapeutic window. The value of the absorption coefficient in this region varies for most tissues from 0.1 cm^{-1} (weak absorption) up to 2 cm^{-1} (strong absorption). This fact justifies the effort to create PSs of second and third generation with absorption peaks in the red and near IR regions of the spectrum.

This phase initiates the damaging effect of PDT which is realised via several pathways: cell necrosis and apoptosis, via lipid peroxydation and protein crosslinking which affect cell membrane enzymes and transmembranous transport; microcirculation arrest, via damage of endothelial cells promotes thrombus formation and vascular stasis which also contributes to tumour ablation and induction of host immune response with release of tumour antigens resulting from inflammation stimulates host immune response even to poorly immunogenic processes.

PDT AGENTS (PHOTOSENSITISERS)

The photosensitiser is a naturally or synthetic compound which undergoes excitation after interaction with suitable radiation emitted from a light source. For the photosensitiser to be particularly efficient for PDT it must have certain characteristics: must be a pure compound; must be soluble in the body's tissue fluids; be able to be incorporated into malignant cells at a much great efficiency than into normal cells; must exhibit strong absorption in the so-called "therapeutic window" (600 – 1000 nm) in which tissue light scattering is low and there is little competing absorption from endogenous molecules; be able to be efficiently fluorescent and produce the singlet oxygen, or other species which can destroy malignant cells; its triplet excited state must be long-lived enough to enable it to photosensitize the production of singlet oxygen; be non toxic for the healthy cells and if possible, it should be quickly expelled from the organisms; must be non-toxic or very low- toxic in the absence of light.

Porphyrins are promising candidates for sensitizing PDT action, because: are typical NIR dyes with red absorption band located in a region of low absorption of tissues; are non-toxic for different healthy cells; are much better incorporated into different kind of human cells than other dyes; are much soluble in different polar or aprotic solvents; due to their aggregation capacity, they could penetrate much better the membrane cells, after this being recovered into their monomer forms. The porphyrins are colored red or purple, fluorescent crystalline pigments, with natural or synthetic origin, having in common a substituted aromatic macrocyclic ring consisting of four pyrrole-type residues, linked together by four methine bridging

groups. Their structure was proposed firstly by Kuster in 1912 and after that by H. Fisher. [26, 27] The general chemical structure for porphyrins as PDT agents are represented in Figure 1.

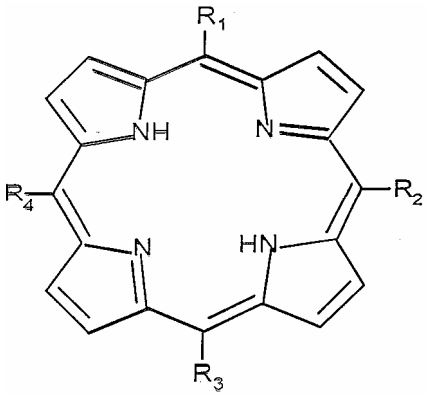

Fig. 1 – The general chemical structure of the porphyrins.

Table 1

The structure of the meso-substituents and the name of the porphyrins

No	R	Porphyrin name	Abbreviation
1.	C_6H_5-H	5,10,15,20-tetra(p-sulfonato)phenyl porphyrin	TPP
2.	C_6H_5-NH_2	5,10,15,20-tetra(4-amino-phenyl) porphyrin	TAPP
3.	C_6H_5-NO_2	5,10,15,20-tetra(4-nitro-phenyl) porphyrin	TNPP
4.	C_6H_5-SO_3H	5,10,15,20-tetra(4-sulfonato-phenyl) porphyrin	TSPP
5.	C_6H_5-OH	5,10,15,20-tetra(4-hydroxy-phenyl) porphyrin	THPP
6.	C_6H_5-CH_3	5,10,15,20-tetra(4-methyl-phenyl) porphyrin	TMPP
7.	C_6H_5-OCH_3	5,10,15,20-tetra(4-methoxy-phenyl)porphyrin	TMOPP
8.	$C_{10}H_7$-H	5,10,15,20-tetra(1-naphthyl) porphyrin	TNP
9.	$C_{10}H_7$-SO_3H	5,10,15,20-tetra(4-sulfonato-naphthyl) porphyrin	TSNP
10.	$C_{10}H_7$-OH	5,10,15,20-tetra(4-hydroxy-naphthyl) porphyrin	THNP

Metallo-porphyrins also have more symmetrical macrocycles than free-base porphyrins, so that Q band spectra generally consists of only two bands, α and β, the former being at longer wavelengths that the latter. The relative intensities of these two bands can be a qualitatively yardstick of just how stable is the metal complexed to the four porphyrinic nitrogen atoms. When α»β, the metal forms a stable square-planar complex with porphyrin (Ni,Pd). Cd, on the other hand, has β»α, and shows a strong instability. The porphyrin efficacy in PDT is due to energy transfer from the porphyrin triplet state to the oxygen ground state and thus depends on porphyrin triplet state characteristics (lifetime and quantum yield), generating singlet oxygen. Singlet oxygen yield is related to triplet quantum yield of the sensitizer and the lifetime of the triplet state. It is expected that the higher the triplet quantum yield with efficient energy transfer, the higher the singlet oxygen yield. Also the longer the lifetime of the triplet state, the higher the amount of singlet oxygen that might be produced since there will be less of the triplet excited state which might be converted back to the ground state. Molecules with long living triplet state cause efficient photosensitization. A long-life triplet state is required for efficient photosensitisation and this criterion may be fulfilled by the incorporation of a dimagnetic metal such as Zn or Al into the porphyrin macrocycle. Metal-free and porphyrins containing paramagnetic metals such as Cu, Co and Fe have a much shorter triplet lifetime and display minimal phototoxicity. Triplet lifetimes are high at porphyrins containing diamagnetic metals such as Mg, Cd, Zn, while paramagnetic ones as Cu,Ni will have short triplet lifetimes.[28] Porphyrins with diamagnetic metals seem to be better suited for photodynamic therapy (PDT) than those with paramagnetic metals because the latter photoinactivate the dyes by shortening the lifetime of the triplet state. In reality the situation is more complex, because even in the metal-free porphyrins free radicals (superoxid anion) can be formed due to breaking of π-bond in the porphyrin matrix. The observed photophysical properties were affected by various molecular aspects, such as extended π-conjugation, structural distortion, and internal heavy atom. The electronic absorption spectrum was red-shifted due to the extended π-conjugation, and the spin orbital coupling was enhanced by the internal heavy atom effect. As a

result of the enhanced spin orbital coupling, the triplet quantum yield increased and the triplet state lifetime was shortened. The incorporation of heavy atoms into the intramolecular structure of the photosensitizer, increases the rate of intersystem crossing and the triplet state quantum yield. [29 - 31]

The quantum yield for singlet oxygen generation, is varied from the free-bases to different metallo-porphyrins, or in different solvents or medium, Table 2.

Table 2

The photophysical properties of the studied porphyrins

Metallo-porphyrins	$\phi(^1O_2)$	Singlet lifetime x 10^{12}(s)	Fluorescence (nm)
MgTNP	0.94	99.7	652
ZnTNP	0.81	103	610
CdTNP	0.91	100	645; 602
CuTNP	0.06	616	-
CoTNP	0.01	500	-
PbTNP	0.15	126	654
PdTNP	0.19	106	670
NiTNP	0.15	638.7	-
AlClTNP	0.89	98	600;676;695;769
$TiCl_2$TNP	0.669	245	838; 931; 988;1019; 1076
$NbCl_3$TNP	0.992	99	610; 660; 700;740
WCl_4TNP	0.88	176	800; 845; 894;960; 1024; 1083
C_2AlTNP	0.907	47.5	614; 666; 731; 960; 1024; 1083
C_8AlTNP	0.793	32.8	676;706 ;826
C_{12}AlTNP	0.685	40.8	706;826;866

The fluorescence quantum yields and lifetimes for a series of metal tetraphenylporphyrins were found to decrease with increasing spin–orbital coupling constant of the central metal ion. In particular, the triplet-state lifetimes decreased in the order Mg > Zn > Pd ≃Cd > Hg and it was possible to relate the rate constants for non-radiative decay processes to the spin–orbital coupling constant of the metal ion although different metal ions interacted with the porphyrin π-system to a different extent.

The ideal properties of a photosensitiser are easily summarised, although the assessment of a sensitiser in these terms is not as straightforward as might be supposed because the heterogeneous nature of biological systems can profoundly affect the properties. Ideally, a sensitiser should be red or near infrared light absorbing; non-toxic, with low skin photosensitising potency; selectively retained in tumours relative to normal adjacent tissue; an efficient generator of cytotoxic species, usually singlet oxygen; fluorescent for visualisation; of defined chemical composition, and preferably water soluble, although with use of liposome delivery systems, the last is not essential.

THE PHOTODEGRADATION REACTION OF PORPHYRINIC AGENTS

It should be noted that the photobleaching does not describe a “simple”photodegradation of the photosensitizer. It includes a chemical modification of the porphyrin, *i.e.* formation of photoproducts. The main factors influencing the photodegradation rate: the meso-substituents; the central metal; the axial ligand attached to the central metal; the aggregation and ionisation processes; the medium temperature; the solvent or binary mixture of solvents.[32-40]

For a low photodegradation rate of the meso-substituted porphyrins must have: a Hammett constant (the substituent constant) equal or less than zero; a small inductive effect from the meso-substituent to the porphyrinic macrocycle; a strong conjugation between macrocycle and meso-substituent. By spectroscopic methods was able to identificate the amidic group, ketonic and peroxidic fragments from the photodegradation products. Mass spectrometry for demonstrating the meso-substituents losing process and the resulted small ketonic compounds

At divalent metallo-porphyrins the photodegradation rate will be more stronger: for metals with empty or completely full d orbital; for the metallo-porphyrins with strong absorption and emission properties; for the metallo-porphyrins with longer lived triplet excited states. The photodegradation mechanism will be superoxid anion one for Cu, Ni, Sn, Co -porphyrins (they have partially filled d orbitals which are able to facilitate the electronic transfer between macrocycle and central metal -yielding to

superoxid anions), a singlet oxygen one for d^0 or d^{10} metals (Zn, Mg, Cd), or a combined one for very fluorescent metallo-porphyrins (as MgP).[41,42]

For trivalent metallo-porphyrins, the photodegradation rate will decrease with the length of axial ligand chain (for example Al(III)-ethyl-TPP will be more easily photodegradated than Al(III)-octyl-TPP); the active molecular fragment will be a μ-oxo-dimer one; the metal is not able to lose the porphyrinic macrocycle.[43] For PDT porphyrins is important to avoid the ketones and peroxides produced during the photodegradation reaction.

In aggregated porphyrins, some photophysical parameters are increased and consequently fluorescence and internal crossing are decreased. Light exposure led to a decrease of the amount of aggregated species. In aqueous solutions, at neutral pH , the electronic absorption spectrum of TSPP is typical of free base porphyrins (D_{2h} symmetry). In the pH range 7-4.5 this porphyrin exists as monocation. The anionic porphyrins (in water or in PBS) can exhibit aggregation processes. In water the sulphonated porphyrins exhibit much complicated, can existing either as dimeric forms or as J-aggregated forms (after $c=2.5 \times 10^{-3}$ M). During the photodegradation reaction, TSPP pass from J-aggregated forms to dimeric forms and finally to monomeric forms which follow the same photodegradation way (excited species, ketones, peroxides, photodegradation products). The mechanism involves the initial formation of dimers, trimers and ultimately the multimer of colloidal dimension which remains stable in solution (H-aggregate or J-aggregate). Sometimes, the H-type intermediate disappears eventually because J-type aggregation is more stable. But, the protonation of the pyrrole nitrogen in the macrocycle is an essential requirement for J-aggregation.[44]

CLINICAL HUMAN APPLICATIONS IN PHOTODYNAMIC THERAPY

The most commonly used method of photodynamic therapy is to administer a photosensitiser, intravenously, orally or by local application to an area of abnormality and allow retention and accumulation in the tissue for a period of time prior to irradiation with appropriate wavelength light, usually from a laser. These externally administered photosensitisers tend to accumulate in rapidly growing tissue, blood vessels and the supporting tissue that grows with malignant tumours. Parenteral administration either by injection or by mouth does produce a period of general photosensitivity and accumulation is in stromal supportive tissue rather directly within growing cells. The problem of targeting the photosensitiser to the rapidly growing cells, and avoiding systemic photosensitisation may be overcome by using endogenous photosensitisation. This involves the exploitation of the increased metabolic activity that may be the 'Achilles heel' of the rapidly growing and dividing cells of cancer. These cells have voracious needs for metabolites; they accumulate the prodrugs required to generate the endogenous photosensitiser to a greater degree than the surrounding tissue. The generated photosensitiser tends to stay within the cells in whose mitochondria it was synthesised. The photosensitiser is activated in tissue using 633–635nm laser light from an appropriate light source, usually a laser.[45]

PHOTODYNAMIC OCCLUSION OF OCULAR NEO-VASCULARIZATION WITH RIBOFLAVIN

A potentially major application of PDT in a non-cancer field is its use in treatment of age-related macular degeneration. This condition, caused by proliferation of neovasculature in the retina, is the major cause of blindness in the over 50s in the western world. Tested on rabbits eyes, angiography revealed immediate and complete vasooclusion, Fig. 2. Histologically vessels were filled with erythrocyte and platelet aggregates. Damage to endothelial cells inclused discontinuity of luminal cytoplasmatic membranes, vacuolization of mitochondria and endoplasmatic reticulum and clumping of nuclear chromatin. Was observed also subconjunctival hemorrhages. First clinical effects were subtle subconjunctival hemorages, chemosis and cyanotic color of the neovascular areas. On the fluorescein angiography are shown the areas of nonperfusion and the occluded neovessels.[46-48]

The fluoroangiography images reveal the necrosis of the tumoral area after only one PDT treatment procedure. After three months the complete atrophy of the tumor and the fluorescence intratumoral disappearance were observed, Figures 2, 3.

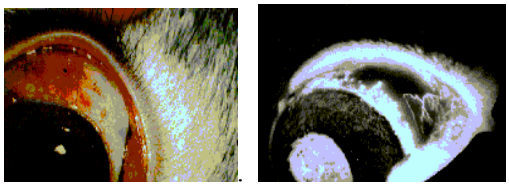

Fig. 2 – Fluorescein angiography before left) and after PDT treatment with Riboflavin on rabbitt (right). Dark area delineates the irradiated sites where hypofluorescence indicates Vascular occlusion.

Fig. 3 – The aspects of ocular tumor in aneritra image and in angiofluorography before PDT (left) and after PDT treatment (right with peritumoral neovascularization) with Riboflavin.

DERMATOLOGICAL APPLICATIONS OF PDT

Research of PDT applications in non-oncological skin disorders primarily concerns psoriasis. Since no uniform protocol was applied in PDT of psoriasis, both systemic and topical sensitization resulted in various responses. In contrast to oncological disorders, PDT of psoriasis requires multiple treatments and lower light doses, because the PDT effect is targeted rather at cell suppression, than at ablation. Vitiligo is a non-contagious pigment disorder. It is characterized by hypo-pigmented patches of skin due to a progressive destruction of melanin pigment producing melanocytes.

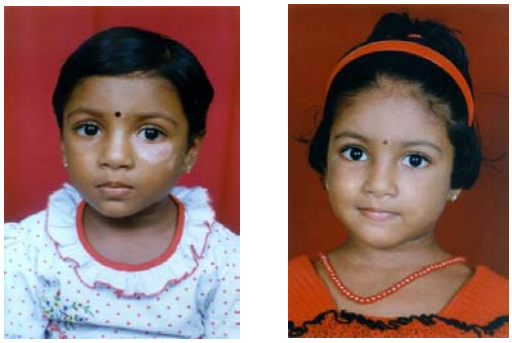

Fig. 4 – The skin of a child with Vitiligo before (left) and after (right) PDT treatment (with authors permission). [49]

After oral administration of khellin in dose of 100 mg followed by exposures to sunlight (15 minutes), was observed repigmentation of vitiliginous skin, Fig. 4 (right).

Furanochromone derivative compounds (especially khellin, Fig. 5) isolated from *Ammi visnaga* are responsible for vitiligo.

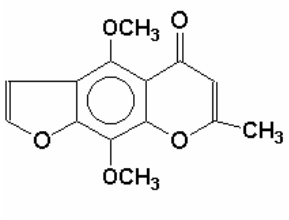

Fig. 5 –The structure of Khellin.

Khellin-UVA photochemotherapy is effective in restoring normal skin color in more than 70% of the originally involved vitiliginous areas in a substantial number of patients with vitiligo after 1 or 2 years of continuous therapy.[50]

HUMAN BRAIN CELLS *in vitro*

Fig. 6 – Photosensitizing drugs (porphyrin as TNP): before PDT treatment (left) and after treatment (right).

The photosensitized effects in biological systems are typically mediated by the triplet state of the sensitizer. That´s why, one of the main condition for a good photosensitizer is that the triplet excited state to be long-lived enough to enable it to photosensitize the production of singlet oxygen. The porphyrinic compounds with long triplet lifetime, will have a high value of the quantum yield of triplet generation.

In vitro samples of brain tumoral tissues were prelevated from human patients and impregnated with different metallo-porphyrin solutions, Figure 6. Photosensitizers preferentially accumulate in malignant tissue whether via increased uptake due to an accelerated cellular proliferation rate, decreased intratumoral pH favoring photosensitizer retention, increased phagocytosis capabilities, leaky vasculature, decreased lymph drainage tumor- associated macrophage engulfing photosensitizers or specific uptake via receptors.[51]

Three consecutive processes occur during the PDT treatment: initial consumption of oxygen through the photodynamic process; pathophysiologic alterations in regional blood supply (hypoxia) and total vascular occlusion (ischemia).

Gross edema and erythema are always the first sign of a PDT response. Cerebral microvasculature, particularly the endothelium is the primary target for PDT. A cytotoxic effect on the vascular network that results in cession of blood circulation and subsequent necrosis of the tissue, could be observed. In experimental tumor systems, clumps of aggregated platelets in the brain parenchyma vessels and focal thrombosis in pial vessels of tumor tissue, may be observed in the microvasculature after light exposure, followed by transient vasoconstriction, vasodilatation and eventual complete blood stasis and hemorrhage, and all these are more pronounced for diamagnetic metallo-porphyrins, because the diamagnetic metal enhance the drug phototoxicity. The blood vessels become dilated and filled with a dense mass of red cells.

CONCLUSIONS

Hence, PDT advantages are as follows:

Photodynamic therapy is applied when surgery is contraindicated because of the tumor spread and serious associated diseases. Photodynamic therapy is targeted at tumor cells, and it causes no damage to healthy tissues. Due to this, when PDT has destroyed a tumor, normal cells begin to propagate and fill the organ's frame.

Photodynamic therapy produces a targeted effect. A photosensitizer is selectively accumulated in a tumor, and it is rapidly eliminated from healthy cells that surround the tumor. Due to this, red light selectively damages the tumor, whereas surrounding tissues remain intact.

Photodynamic therapy avoids the systemic effect on the human being (in the case of chemotherapy of tumors, this effect does take place). Photodynamic therapy treats a region exposed to light. As a result, the patient is not subjected to an unwanted systemic effect. This makes it possible to prevent the patient from all side effects, typical of chemotherapy (such as nausea, vomiting, stomatitis, loss of hair, and inhibition of hematopoiesis).

Photodynamic therapy is cost-effective. For a majority of patients, PDT is a noninvasive or minimally invasive method. It is also a tolerant, local, and inexpensive technique, which can treat a

variety of malignant tumors (primary, relapsing, and metastatic).

Photodynamic therapy is a beautiful concept, presenting the possibility that a light driven reaction can be exploited to destroy diseased tissue. It now has an established role in the treatment of eye and skin disease. Thus photodynamic therapy has been initially used for the treatment of desperate and very advanced cancers were other therapies have failed the patient.

It is now becoming clear that if cancers are found at an early stage, local minimally invasive targeted therapy such as photodynamic therapy may be very successful. This avoids mutilating surgery and the inevitable normal tissue damage and complications associated with other therapies. Clinicians are now actively seeking and screening for early cancers or pre-cancerous changes. It is essential that the treatment offered these patients is not worse than the disease itself, which when detected may be causing no symptoms but has the potential to be lethal if progress is not interrupted. There is no doubt that the long scientific efforts of photodynamic therapy will allow useful patient treatments in the future.

REFERENCES

1. R. M. Ion, *Curr. Top. Biophys.,* **2000,** *24,* 30-42.
2. D. Frackowiak, A. Planner and R. M. Ion, in "Near-infrared dyes for high technology applications", S.Daehne, U.Resch-Genger, O.Wolfbeis (Eds.) NATO ASI SERIES, Vol. 3/52, Kluwer Academic Publishers, Dordrecht/Boston/London, 1998, p. 87-114.
3. R. L. Lipson, E. J. Baldes and A. M. Olsen, *J. Nat. Cancer Inst.*, **1961**, 26, 1-8.
4. H. B. Gregorie, E. O. Horger and J. L. Ward, *Ann. Surg.,* **1968**, 167, 820-828.
5. R. L. Lipson, M. J. Gray and E. J. Baldes, *Proc. 9-th Int.Cancer Congress*, Tokyo, Japan, 1966, p. 393.
6. I. Diamond, A. F. McDonagh and C. B. Wilson, *Lancet.*, **1972**, 1175-1177.
7. J.F.Kelly and M.E. Snell, *J. Urol.*, **1976**, 115, 150-151.
8. T.J. Dougherty, *Photochem. Photobiol.*, **1987**, 46, 569-572.
9. T.J. Dougherty, *Sem. Surg.Onc.*, **1989**, 5, 6-16.
10. T. J. Dougherty, R. E.Thoma, D. Boyle and K. R. Weishaupt, in "Laser in Photomedicine and Photobiology", R. Pratesi, C.A. Sacchi (Eds)., New-York, Springer, **1980**, 67-75.
11. D. Kessel, P. Thompson, B. Musselman and C. K. Chang, *Photochem. Photobiol.,* **1987**, 46, 563-567.
12. T. J. Dougherty, J. E. Kaufman and A. Goldfarb, *Cancer Res*., **1978**, 38, 2628-2635.
13. P. J. Bugelski, C. W. Porter and T. J. Dougherty, *Cancer Res.*, **1981**, 41, 4606-4612.
14. S. Amyles*, JAMA*, **2001**, 285, 2317-2318.
15. M. Wainwright, *J. Antimicrob. Chemother.*, **1998**, 42, 13-28.
16. C. Abels and A. E. Goetz, in "The Fundamental Bases of Phototherapy", H. Honigsmann, G. Jori, A. R. Young (Eds), OEMF spa - Milano, 1996, p. 265-284.
17. Y. Hayata, H. Kato and C. Konaka, *Chest.*, **1982**, 81, 269-277.
18. R. M. Ion, A. Planner, K. Wicktowicz and D. Frakowiak, *Acta Biochimica Polonica,* **1998,** 45, 833-845.
19. R. M. Ion, in "Porfirinele si terapia fotodinamica a cancerului", FMR Ed., Bucureşti, 2003, Cap.8, p.113-145.
20. D. Frackowiak, A. Planner, A. Waszkowiak, A. Boguta, H. Manikowski, R. M. Ion and K. Wiktorowicz, *J.Photochem.Photobiol., A:Chem.,* **2001**, 141, 101-108.
21. R. R. Allison, G. H. Downie, R. Cuenca, X. Hu, C. J. H. Childs and C. H. Sibata, *Photodiagnosis and photodynamic therapy* **2004,** 1, 27-35.
22. R. M. Ion, *J.Biomed Optics,* **1999,** 4, 319-325.
23. R. M. Ion, in "Materiale Nanocristaline", FMR Ed., Bucureşti, 2003, p.111-139.
24. R. Alexandrova, R. M. Ion, E. Stoykova and S. Shurilinkov, *Proc. SPIE,* **2003***,* 5226, 423-428.
25. R. Alexandrova, O. Sabotinov, E. Stoykova, R. M. Ion, S. Shurilinkov and G. Minchev, *Proc.SPIE,* **2004**, 5449, 227-234.
26. W. Kuster, *Z. Physiol. Chem.,* **1913**, 82, 463-483.
27. H. Fisher, "Die Chemie des Pyrrols", Liebeg (Eds.), 1940, Vol.2, p. 28-45.
28. D. Frackowiak, A. Waszkowiak, R. M. Ion, K. Wiktorowicz, I. Cofta and H. Manikowski, *Acta Biochimica Polonica,* **2001**, 48, 257-269.
29. R. Alexandrova, E. Stoykova and R. M. Ion, *Exp. Pathol. Parasithol.,* **2005,** 7, 7-23.
30. R. W. Boyle and D. Dolphin, *Photochem. Photobiol*., **1996**, 64, 469-485.
31. J. M. Assour and S. M. Harrison, *J. Phys. Chem.* **1964,** 68, 872-876.
32. R. M. Ion, *Oftalmologia* **2003,** XLXIV, 77-82.
33. R. M. Ion, *Rev.Chim*. (Bucharest), **1993**, 44, 957-962.
34. R. M. Ion, *Rev.Chim.* (Bucharest), **1995**, 46, 134-142.
35. R. M. Ion and G. A. Petre, *Rev.Chim.* (Bucharest),**1996,** 47,113-119.
36. R. M. Ion and C. Mandravel, *South. Braz. J. Chem. Soc.,* **1996***,* V, 111-117.
37. R. M. Ion, *Rev.Chim*. (Bucharest), **1994**, 9, 789-791.
38. R. M. Ion, L. Teodorescu, C. Mandravel, E. Volanski and M. Hilebrand, *Rev. Chim.* (Bucharest), **1990,** 41, 129-134.
39. L.Teodorescu and R. M. Ion, *Rev.Chim.* (Bucharest), **1990**, 41, 312-318.
40. R. M. Ion and C. Mandravel, *Rev. Chim.* (Bucharest), **1993**, 44, 61-70.
41. R. M. Ion and M. S. Tataru, *Rev.Chim.* (Bucharest), **1993**, 44, 736-739.
42. R. M. Ion, *Rev. Chim.* (Bucharest), **1993**, 44, 431-435.
43. R. M. Ion and S. Apostol, *Rev. Chim.* (Bucharest), **2005**, 56(6) 607-620.
44. R. M. Ion, *Rev.Chim.* (Bucharest), **1994**, 45,321-324.
45. C. Constantin, M. Neagu, G. Manda, R. M. Ion and D. Iordachescu. *Rom. Arch. Microbiol. Immunol.,* **2004,** 63,159-168.
46. M. A. Ionita, B. Carstocea, R. M. Ion and V. I. R. Niculescu, *Oftalmologia,* **2002,** XLXIV, *82-86*.
47. R. M. Ion, M. Ionita and B. Carstocea, *Oftalmologia***, 2003,** XLIX, 29-35.
48. R. M. Ion, M. A. Ionita and B. Carstocea, *Rev. Chim.* (Bucharest), **2006,** 57, 723-740.
49. A. M. El-Mofy in "Vitiligo and Psoralens", Oxford, Pergamon Press, 1968, p. 147-125.
50. K. Gunaydin, G. Topcu and R. M. Ion, *Nat. Prod. Lett.,* **2002,** 16, 65-70.
51. M. L. Pascu, L. Danaila, R. M. Ion, A. Popescu and M. Pascu, *Proc.SPIE,* **2000**, 4068, 712-720.