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Photodynamic therapy: mechanism of action and ways to improve the efficiency of treatment

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Summary. Photodynamic therapy is treatment modality involving the administration of photosensitizing compound, which selectively accumulates in the hyperproliferative target cells followed by local irradiation with visible light of lesion. Eventually target tissue will be damaged by necrosis and apoptosis. Action of treatment is described from absorption of light till damage of tissue. Several rationale proposals to increase the efficiency of described treatment modality are suggested: to evaluate the antiproliferative activity of new coming photosensitizers, to combine photosensitization with other treatment modalities in molecular level, exploring mechanism of apoptosis, to increase the efficiency of treatment by combination with ionizing radiation, hyperthermia or ligation of peripheral benzodiazepine receptors.

Introduction

Photodynamic therapy is an entirely new treatment modality and its development can be likened to that of the discovery of antibiotics. This is just beginning, and its possible uses are only limited by the imagination.

(McCaughan J. S. Drugs & Aging 1999;15:49–68.)

The first attempts to use photosensitizing drugs for the cure of skin diseases dates back to ancient Egypt, India, and Greece, where psoralen-containing plant extracts and light were applied to treat psoriasis and vitiligo. The term *photodynamic* was coined by Von Tappeiner in 1904 to describe oxygen-dependent chemical reactions induced by photosensitization. In general, photosensitization-based therapy (PDT) is a treatment modality involving the administration of a photosensitizing compound, which selectively accumulates in the target cells, followed by local irradiation of the lesion with visible light. The combination of two absolutely nontoxic elements, i. e. drug and light, in the presence of oxygen results in the selective destruction of tissue. The tumor-accumulating property of porphyrins was revealed by Policard. He found the characteristic red fluorescence of neoplastic, embryonic, and traumatized tissues after the application of hematoporphyrin.

The expanding use of PDT is based on the pioneering work of T. J. Dougherty (1), who presented extensive data on the successful application of this novel technique for the treatment of cancer in 1978. Since then, PDT has gained increasing interest in medicine, representing an experimental tool for the detection and treatment of tumors located in the lung, esophagus, colon, peritoneum, pleura, genitourinary tract, brain, eye, and skin. Intensive clinical research culminated in the approval of PDT for the management of selected malignancies in Canada, Japan, France, the Netherlands, Germany, and the United States (2).

The main advantages of PDT over other for instance oncotherapies include rather significant degree of selectivity of drug accumulation in the tumor tissue, the absence of systemic toxicity of the drug alone, the ability to irradiate only tumor, the possibility of treating multiple lesions simultaneously and the ability to retreat a tumor in order to improve the response.

Moreover, numerous investigations demonstrate possible practical usefulness of photosensitization in the broad field of different sciences, diverse conditions, such as dermatological diseases, atherosclerosis, infectious diseases, rheumatoid arthritis, age-related macular degeneration, restenosis, AIDS, hematological diseases may be successfully treated by photosensitization.

Thus, question arises inevitably: how does photodynamic therapy work?

Three indispensable components of photosensitization

It is a result of the combined effect of three non-toxic agents – photosensitizer, light and oxygen, thus it is necessary to describe all of them separately.

Photosensitizers

A large number of photosensitizing drugs have been tested *in vitro* and *in vivo* during last 10 years. Table 1 presents most commonly used photosensitizers and precursors in photodynamic therapy (7).

The physico-chemical properties of the photosensitizer are very important for the efficacy of photosensitization. Chemical purity, capability to localize specifically in neoplastic tissue, short time interval between the administration of the drug and its maximal accumulation in hyperproliferating tissue, rapid clearance from normal tissues, activation at wavelength with optimal tissue penetration, high quantum yields for the generation of singlet oxygen, and lack of dark toxicity are desirable features of an ideal photosensitizer. The fundamental prerequisite for optimal response to photosensitization is a sufficient amount of drug localized in the target tissue. Initially, photosensitizers are taken up by most normal and hyperproliferating cells, but are retained longer in the last one (3). The mechanisms of this selective prolonged retention are not understood in detail. Increased blood vessel permeability as well as poor lymphatic drainage in neoplastic tissues may contribute to the retention of the drug in neoplastic lesions.

Hematoporphyrin derivative (HPD) was the first systematically studied photosensitizer for clinical PDT. The other one – Photofrin (PII) – has several absorption peaks that can be useful in “diagnostic mode”.

Unfortunately, significant tissue penetration is achieved by light at 630 to 635 nm, which corresponds to the weakest absorption of PII. Moreover, the coetaneous accumulation of porphyrin-based photosensitizing drugs and their slow clearance from the skin leads to long-lasting skin photosensitivity, requiring one to avoid light from 4 to 6 weeks after photosensitization (4).

Light sources

Initially, photosensitization has been performed with the use of conventional gas discharge lamps. The introduction of lasers equipped with optical fibers revolutionized photosensitization and expanded its

Table 1. Photosensitizers and their precursors used in experimental and clinical photodynamic therapy applications

Porphyrins Hematoporphyrin derivative Dihematoporphyrin ether/ester Porfimer sodium Tetrasodium-meso-tetraphenylporphyrin-sulphonate Metallotetra-azaporphyrin
Porphyrin precursors δ-Aminolevulinic acid (ALA) δ-Aminolevulinic acid (ALA)-methyl-, propyl-, hexyl-esters
Phthaloalocyanines Chloroaluminum tetra-sulfonated phthalocyanine Zinc(II)phthalocyanine Silicone naphthalocyanine Aluminum sulfonated phthalocyanine
Porphycenes 9-Acetoxy-2,7,12,17-tetra-N-propylporphycene 2-Hydroxyethyl-7,12,17-tris(methoxyethyl)porphycene 23-carboxy-24-methoxycarbonylbenzo(2,3)-7,12,17-tri(methoxyethyl)-porphycene
Chlorines Monoaspartyl chlorine e ₆ , diaspartyl chlorine e ₆ Chlorine e ₆ sodium, bacteriochlorin a Benzoporphyrin derivative monoacid ring A
Pheophorbides Pheophorbide a, bacteriopheophorbide
Others Fluoresceins (fluorescein sodium, tetrabromofluorescein-eosin) Anthracenes (anthraquinone, acridine orange, yellow) Hypericin Furocoumarin (5-methoxypsoralen, 8-methoxypsoralen) Chlorophyll derivatives Purpurins (metallopurpurin, tin etiopurpurin Sn ET2) Phenothiazines Methylene blue, violet green Azure C, thionine, Nile blue A Hypocrellin Rose Bengal Rhodamine 123 Lutetium texaphyrin

applicability in medicine, enabling the endoscopic delivery of light to almost every site of the human body. Photodynamic treatment in dermatology is simplified by the accessibility of the skin to light application and allows to use any light source with the appropriate spectrum. Metal halogen lamp, which emits 600 to 800 nm radiation at high power density, short-arc xenon lamp, tunable over a bandwidth between 400 and 1200 nm. The broad light beam produced by incoherent lamps is useful for the treatment of large lesions.

In contrast to traditional incandescent lamps, lasers provide the exact selection of wavelengths and the precise application of light. Pulsed lasers, such as the gold vapor laser (GVL) and the copper vapor laser-pumped dye laser (GVDL), produce brief light pulses of millisecond to nanosecond duration (5). The comparison of continuous-wave and pulsed lasers in practice has shown no difference. Tunable solid-state lasers, such as the neodymium: YAG laser, are particularly useful for PDT. The above-listed laser systems are expensive, relatively immobile, and require frequent repair. The development of semiconductor diode lasers is a novel approach to circumvent these disadvantages. Portable diode lasers, such as the gallium-aluminium-arsenide laser, produce light in the range from 770 to 850 nm, which corresponds to the absorption peaks of many new photosensitizers.

Oxygen

Numerous investigations supported the idea that the efficacy of photosensitization is directly related to the yield of $^1\text{O}_2$ in the tumor environment and the yield of $^1\text{O}_2$ depends on the concentration of oxygen in the tissue (3). Hypoxic cells are very resistant to photosensitization and the photodynamic reaction mechanism itself may consume oxygen at a rate sufficient to inhibit further photosensitization effects. It has been suggested, that hyperbaric oxygen might enhance the photosensitization effect.

“Photosensitization dose” measurements

As photosensitization is the result of the combined effect of three agents – photosensitizer, light and oxygen – measurement of each component is required for an ideal “photosensitization dose” evaluation. However, limitations in measuring each agent limit the assessment of the true photosensitization dose.

By no means, in order to optimize photosensitization, it is important to know the photosensitizer pharmacokinetics and concentration in the normal and tumor tissues. The most reliable method available to determine the photosensitizer concentration requires

continuous sampling of the blood serum and tissue biopsies. The obvious limitations in taking multiple blood and tissue biopsies from patients has stimulated researchers to develop non-invasive systems capable of measuring photosensitizer concentration using its fluorescence properties (6). Although not yet perfected, these new non-invasive techniques will eventually permit more individualized photosensitizer concentration measurements.

The successful eradication of target tissue requires a sufficient concentration of photosensitizer within it and the presence of photoactivating light in the malignant cells. The penetration of light through the tissue depth is dependent on the characteristics of the treated tissue and on the wavelength of the light. Besides, the light penetration is limited by optical scattering within the tissue, the absorption by endogenous chromophores, and the absorption of light by the sensitizing drug (self-shielding).

The light dose regimens used in practice vary widely depending on the location, size, and histopathological type of the lesion. Surface treatment with Photofrin and 630 nm light requires fluencies ranging from 25 to 300 J/m².

Cell and tissue destruction induced after photosensitization

Positive clinical results involving PDT have led to expanded desire to identify molecular, cellular and tumor response associated with this treatment. Biochemical studies, performed over the past 15–20 years have provided a plethora of information on molecular, cellular and tumor PDT targets.

Photochemistry

The initiating step of the photosensitizing mechanism is the absorption of a light photon by the sensitizer, causing a promotion of the drug molecule from its ground state to the extremely unstable excited singlet state with a half life in range of 10^{-6} to 10^{-9} seconds (Fig. 1). The singlet excited photosensitizer either decays back to the ground state, resulting in the fluorescence or undergoes intersystem crossover to the longer lived (10^{-3} second) triplet excited state. Tumor destruction is most efficient using compounds with a long triplet half-life and a high quantum yield for the triplet excited state. The interaction of the triplet sensitizer with surrounding molecules results in two types of photo-oxidative reaction (Fig. 1).

Type I pathway involves electron or hydrogen atom transfer, producing radical forms of the photosensi-

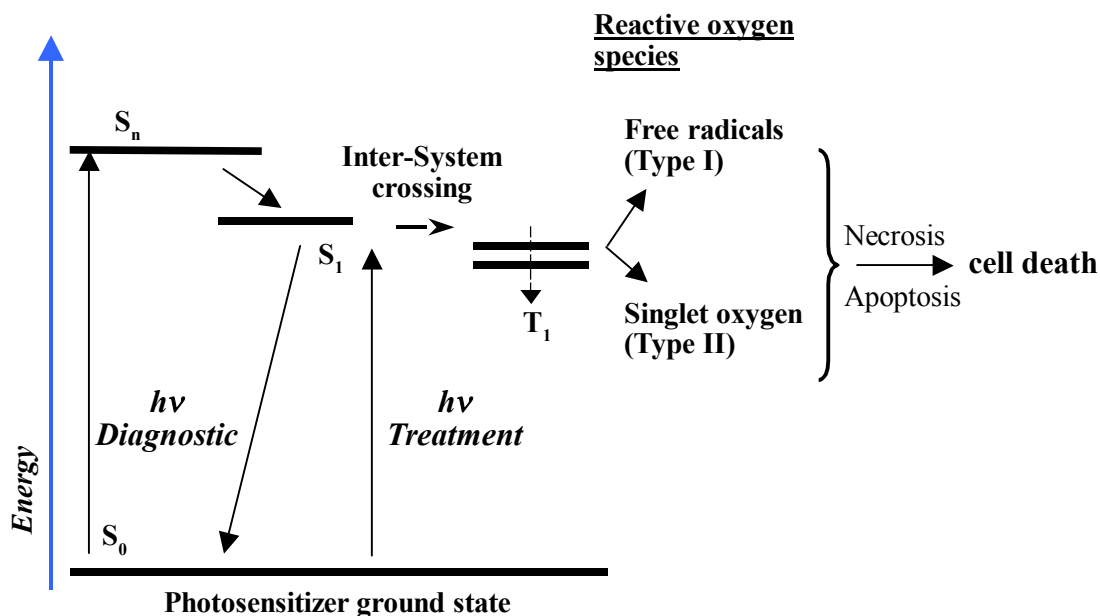


Fig. 1. Jablonski's energy level diagram for photodynamic therapy (adapted from C. H. Sibata et al, 2001 (4))

$h\nu$ Diagnostic: diagnosis excitation photon; $h\nu$ Fluorescence: energy of fluorescence photons; $h\nu$ Treatment: treatment excitation photon; S_0 : photosensitizer ground state; S_1 and S_n : photosensitizer excited singlet states; T_1 : photosensitizer excited triplet state.

tizer or the substrate. These intermediates may react with oxygen to form peroxides, superoxide ions, and hydroxyl radicals, which initiate free radical chain reactions. Type II mechanism is mediated by the energy transfer process with ground state oxygen (1O_2) and to the return of the sensitizer to his ground state. The *in situ* generation of singlet oxygen via type II pathway appears to play the central role in photodynamic cytotoxicity because of the highly efficient interaction of the 1O_2 species with different biomolecules.

Uptake and localization of photosensitizers in the tumor cells

Why are the cellular sites of photosensitizer localization and photodamage so important? To facilitate drug development, it is often necessary to identify a target. A systematic study of structure-activity relationships can then help in improving the therapeutic procedure.

Unfortunately, clinically accepted PII contains several porphyrin components with different lipophilicity and supposedly different intracellular localization. Since the second-generation sensitizers tend to be more pure compounds, loci of localization can often be easily identified. Mitochondria, lysosomes, plasma membrane, endoplasmic reticulum have been evaluated as potential PDT targets in the tumor cells.

What factors do determine specific localization of photosensitizer in the cells? Its worldwide accepted, that sensitizer's lipophilicity, aggregation degree mostly determine the accumulating efficiency and localization specificity in the tumor cells (7).

After intravenous (i. v.) administration of hydrophobic sensitizers, the compounds are, in general, strongly bound to lipoproteins (high density lipoproteins (HDLs) and low density lipoproteins (LDLs)), distributed within the blood system and transported to the malignant tissue with a distinct selectivity. It is well accepted today that the tumor selectivity increases to some extent with the lipophilic character of the sensitizing agent (8).

The preferred accumulation of lipophilic sensitizers, within tumor tissues is in reasonable harmony with the observation that neoplastic cells express a particularly large number of LDL membrane receptors (9). Following receptor-mediated endocytosis, the sensitizer molecules preferentially accumulate in the lipophilic compartments of tumor cells, including plasma, mitochondrial, endoplasmic reticulum, nuclear and lysosomal membranes.

However, it should be clearly emphasized that other factors, such as lower tumor pH, also correlate with an enhanced uptake of photosensitizers. The point is, that low pH value of most tumors is related to their poor oxygen supply and high glycolytic activity. After i. v. injection of hydrophilic photosensitizers, the drugs

are largely carried by albumin and other serum proteins (10). Microscopic measurements reveal a preferred accumulation of these sensitizers within the interstitial space and the vascular stroma of the tumor tissue. Due to their hydrophilic character, the tendency to diffuse across the plasma membrane into the cytoplasm is small.

As outlined above, the site of the primary localization of the sensitizer strongly depends on the lipophilic or hydrophilic character of the drug considered.

In general, hydrophobic drugs attack the tumor cells mainly by direct interactions. In contrast, water-soluble sensitizers kill hyperproliferating cells indirectly by damaging blood vessels and interrupting the supply of oxygen and other essential nutrients.

PDT induced cell damage

Because of limited migration of 1O_2 from the site of its formation, sites of initial cell or tissue damage of photosensitization are closely related to the localization of the sensitizer.

In practice, sensitizers tend not to accumulate in cell nuclei. So, photosensitization has generally a low potential of causing DNA damage, mutations and carcinogenesis.

PDT damage of plasma membrane can be observed within few minutes after light exposure. This type of damage is manifested as swelling and blebbing, shedding of vesicles containing plasma membrane marker enzymes, cytosolic and lysosomal enzymes, reduction of active transport, depolarization of plasma membrane, inhibition of activities of plasma membrane enzymes such as Na^+K^+ – adenosine triphosphatase (AT-Pase), a rise in Ca^{2+} , up- and down- regulation of surface antigens, etc. (7).

Cellular membranes, including plasma, mitochondrial and sometimes nuclear membranes, are severely damaged by oxidation of unsaturated fatty acid residues and of cholesterol (11). The capacity of liberated lysosomal enzymes, especially of neutral proteinases, to damage tumor cells has been documented and will not be discussed further. Nevertheless, the hypothesis that the liberation of lysosomal enzymes is generally a cofactor in the initiation of necrotic cell death remains to be explained.

The examination of the mechanism of cell lysis indicates that oxidative damage of membrane transport proteins induces a rapid derangement of ionic homeostasis of Na^+ , K^+ , Ca^{2+} , etc., while membrane integrity is still retained at this time. The release of all these

physiologically active ions has been associated with an immediate induction of acute inflammatory reactions. The pathophysiological processes mediated by an increased cytosolic Ca^{2+} concentration are summarized below. For example, Ca^{2+} catalyses the release of arachidonic acid through an allosteric activation of phospholipase A_2 , induces the protein kinase C-dependent generation of superoxide radicals (O_2^-) and leads to a condensation of chromatin around the nucleus (12). The concurrent activation of endonuclease for DNA fragmentation and the effect of the inhibitors of protein kinase C and calmodulin provide evidence that the signals responsible for the initiation of PDT-induced apoptosis might be transduced by Ca^{2+} (12). The formation of intravascular thrombi is also catalyzed by Ca^{2+} through the breakdown of prothrombin and the liberation of thrombin fragments. Recent experiments clearly demonstrate that an increase in cytosolic Ca^{2+} stimulates the release of the “von Willebrand factor” (vWf) (13) – an adhesive glycoprotein synthesized by endothelial cells, which mediates the adhesion of platelets to the injured vessel walls.

The breakdown of cellular membranes causes a liberation of phospholipids, which are readily attacked by phospholipases and degraded to free fatty acids. Of major importance is the release of arachidonic acid. Whilst cyclooxygenase, a multi enzyme complex, catalyses the conversion to thromboxanes (A_2 , B) and prostaglandins (PGD_2 , PGE_2 , 6-keto $PGF_{1\alpha}$), 5-lipoxygenase converts arachidonic acid to leukotrienes (B_4 , C_4 , D_4) and hydroxy acids. Together, the generated substances initiate an acute inflammatory reaction. Prostaglandins primarily cause a vasodilatation of terminal arterioles and damage the endothelial and smooth muscle cells, which coat the inner surface of blood vessels (14). The gaps between endothelial cells lead to local edema by an enhanced efflux of water, macromolecules and blood cells, i. e. mast cells, neutrophils, leukocytes and macrophages, into the tumors tissue. Thromboxanes and leukotrienes harm endothelial cells by arteriolar and venular vasoconstrictions. Moreover, thromboxanes promote the aggregation of platelets and trigger the formation of intravascular thrombi. The stasis of the blood flow finally creates areas of local hypoxia and initiates necrotic processes by nutrient deprivation. In response to the extraordinary rapidity with which the platelets aggregate and adhere to damaged tissue, the contents of their dense granules and discharged and various biologically active substances, such as histamine and bradykinin, are released and augment the pathological effects of the eicosanoids.

Cell death pathways induced after photosensitization

With increasing recognition of photosensitization as an efficacious treatment, there is also increased interest in elucidating the mechanisms by which it causes the death in the cells and tissue – in order to enhance this destructive action on target tissue and optimize therapeutic strategies.

Two distinct modes of cell death after photosensitization – apoptosis and necrosis – can be recognized based on differences in the morphological, biochemical and molecular changes of dying cell. The most common feature of apoptosis is active participation of the cell in its self-annihilation. The cell mobilizes a cascade of events that leads to its disintegration and the formation of “apoptotic bodies” which are subsequently phagocytized by the neighboring cells without involving inflammation (12). Increased cytoplasmic Ca^{2+} concentration, cell dehydration, chromatin condensation originating at the nuclear periphery, activation of endonuclease which has preference to DNA at the internucleosomal (linker) sections, proteolysis, fragmentation of the nucleus and fragmentation of the cell are the most characteristic events of apoptosis. On the other hand, even during advanced stages of apoptosis, the structural integrity and the transport function of the plasma membrane are preserved. Also preserved and functionally active are the mitochondria and lysosomes. So, apoptosis looks like “black hole” of cell death: it draws everything inward and nothing escapes its biochemical event horizon (15).

Necrosis is an alternative to the apoptotic mechanism of cell death. Most often it is induced by an overdose of cytotoxic agents. While apoptosis requires active participation of the whole cell, necrosis is a passive and degenerative process. *In vivo*, necrosis triggers the inflammatory response in the tissue. In contrast, remains of apoptotic cells are phagocytized not only by the “professional” macrophages, but also by neighboring cells, without evoking any inflammatory reaction. The early event of necrosis is swelling of cell, followed by rupture of the plasma membrane and release of the cytoplasmic content (12).

Apoptotic process can be divided into three phases: activation, propagation and execution.

The apoptotic machinery can be divided into two classes of components: sensors and effectors. The sensors are responsible for monitoring the extracellular/intracellular environment for conditions to live or to die. They are involved in “activation” phase.

Extracellular sensors include cell surface receptors that bind survival or death factors and might normally limit the size of cell population (“cell murder”). In addition, intracellular sensors activate the death pathway in response to detecting irreparable DNA damage, signaling imbalance provoked by oncogene action, survival factor insufficiency or hypoxia (“cell suicide”). Many of the signals that elicit apoptosis converge on the mitochondria, which respond to proapoptotic signals by releasing cyt_c (Fig. 2).

Members of Bcl-2 family of proteins (proapoptotic – Bax, Bak, Bid, Bin; antiapoptotic – Bcl-2, Bcl-X_L, Bcl-W) regulate death signaling through cyt_c release.

The ultimate effectors (active in “propagation” phase) of apoptosis include intracellular proteases termed caspases. Two “gatekeepers” caspase-8 and -9, are activated by death receptor such as Fas or by cyt_c respectively. These proximal caspases trigger the activation of a dozen or more effector caspases that execute the death program through selective destruction of subcellular structures and genome.

Bcl-2 was found to be a protooncogene that blocks apoptosis. Now it is known, that Bcl-2 belongs to a large protein family containing death antagonists (Bcl-2, Bcl-X_L, Bcl-w, Bfl-1, Bcl-1, Mcl-1) and death agonists (Bax, Bak, Bcl-X_S, Bad, Bid, Bik, Hrk) (14). It has been assumed, that the ratio of death antagonists to agonists in a cell may determine whether a cell responds to an apoptotic signal.

The morphological manifestation of apoptosis (“execution” phase) can be ascribed as degradation of various structural proteins and DNA, as showed in Fig. 3.

This degradation process was executed by several proteins, which were activated by specific caspases. Other pathways may also be activated and many other gene products are known to be up regulated, however which gene belongs to what delayed apoptotic mechanism and which genes belong to what survival mechanism remains to be determined. Two “points of no return” have been found in apoptosis processing. One of point of-no-return involves the opening of the mitochondrial megapore at the “S” site (16); other involves the pore formation at “P” site.

The mitochondrial megapore is comprised of a large complex of proteins that spans in outer and inner membranes: benzodiazepine receptor (protoporphyrin IX, PpIX is a ligand), voltage-dependent anion channel (VDAC), adenine nucleotide transporter (ANT) and cyclophilin D. The mitochondrial megapore can be inhibited by Bcl-2 or Bcl-X_L (17), however once either AIF is released from “S” site or cyt_c is released from “P” site cell cannot be rescued from death (15).

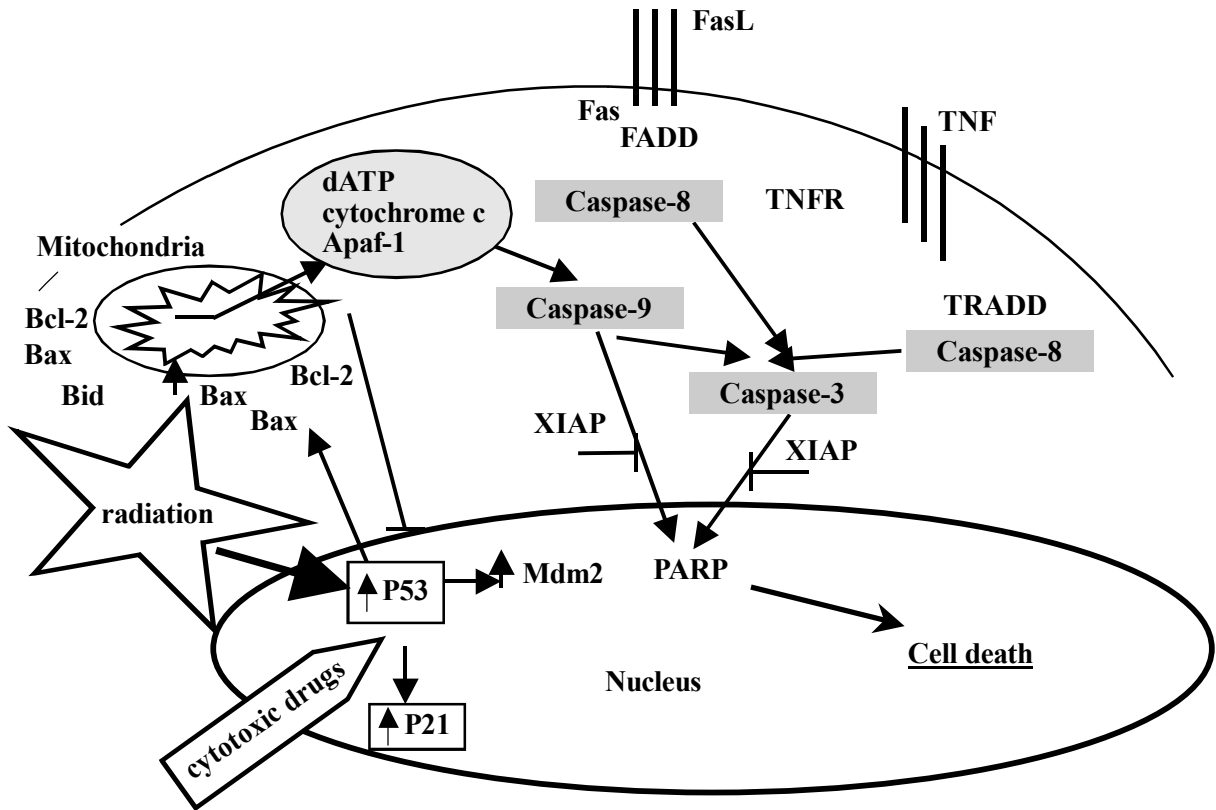


Fig. 2. A simplified schematic diagram showing p53-dependent and independent apoptotic pathways (modified after Bentley and Pepper, 2000)

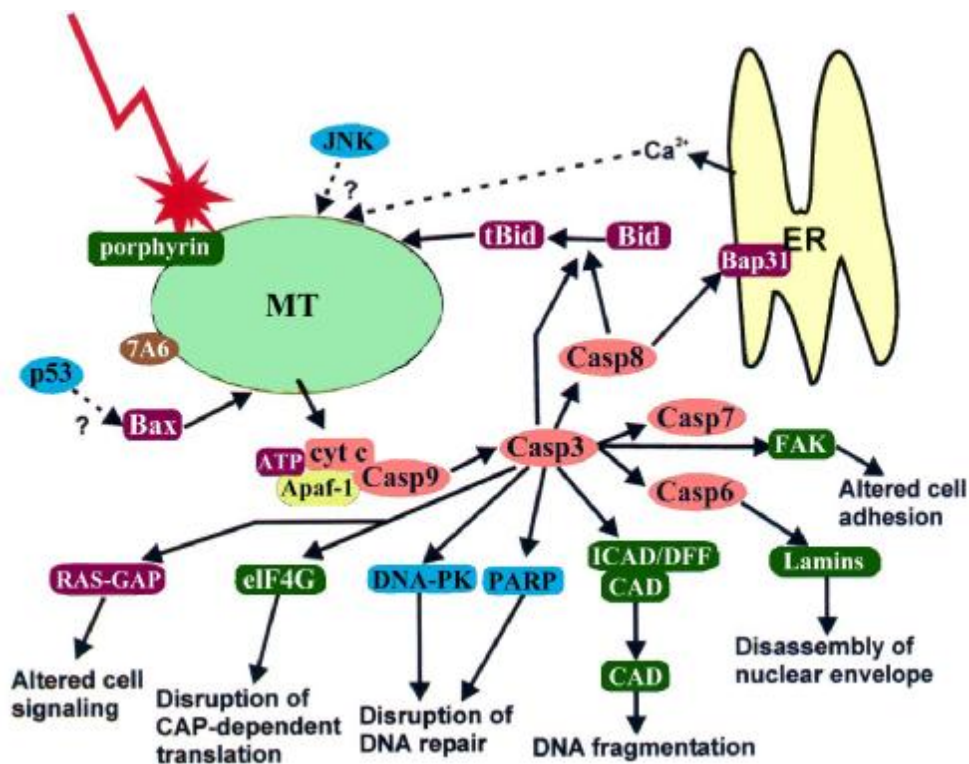


Fig. 3. PDT with porphyrin-derived photosensitizers can include rapid apoptosis due to their close association with mitochondria (modified after Granville et al, 2001)

Apoptosis is both positively and negatively regulated. In addition to the numerous factors involved in the induction of apoptosis many other factors have been identified that prevent cell death. Anti-apoptotic ligands include growth factors and cytokines many of which induce anti-apoptotic Bcl-2 family members. Apoptosis inhibitors include factors that inhibit caspases directly or prevent their activation. Interestingly, TNF- α has antiapoptotic effects in addition to its pro-apoptotic effects. It can activate the transcription factor NF- κ B that then induces the expression of IAP, an inhibition of caspases 3, 7, 9. Overall, apoptosis is a complex process involving numerous factors operating in multiple pathways that must be carefully regulated for the proper growth of organism. Failure to properly execute apoptosis can lead to a number of diseases, including cancer.

Photosensitization induced tissue damage

The mechanism of transport and localization of sensitizers in for instance tumor tissues is not well understood. A solid tumor contains, in addition to neoplastic cells, the vascular and interstitial compartments. Although the tumor vasculature originates from the host vasculature, its organization may be completely different in manners depending on the tumor type, its growth rate, and its location. Its morphology suggests that the vascular permeability of tumors is significantly higher than that of normal tissues and that the vasculature is vulnerable. It has been suggested that hydrophilic dyes are mainly transported by albumin and globulins and distributed in the vascular tumor stromal tissue. More hydrophobic drugs are preferentially incorporated into lipoproteins and localized in neoplastic cells.

Data obtained on the intratumoral localization of dyes have indicated that there are two main types of tumor destruction: 1) *indirect killing of tumor cells*, whereby initial destruction of the vascular system (endothelium and other components of vascular wall) and intercellular matrix is followed by hypoxia, which finally results in death of the neoplastic cells in tumors; 2) *direct killing of tumor cells*, whereby tumor cells are damaged by the direct effect of photosensitization as a consequence of injure of tumor cell.

However, there is increasing body of evidence that PDT-induced tumor damage is primarily due to vascular damage inducing hypoxia of tumor cells and tumor necrosis.

Also, initial congestion (hyperemia), plasma exudation and stasis followed by edema, extravasation of erythrocytes, and infiltration of white blood cells due

to a marked increase in vascular permeability or rupture of vascular walls are seen in HpDe-induced PDT (18). Recently, an immune response has been shown to be involved in PDT-induced effects in neoplastic tissues. For instance, tumor-infiltrating macrophages were found to take up more Photofrin, AIPcS₂, and AIPcS₄ than did neoplastic cells and to release tumor necrosis factor (TNF) after Photofrin-PDT *in vitro* (19). This factor may contribute to tumor regression by direct inactivation of tumor cells or by mediating hemorrhagic necrosis (19). Furthermore, photosensitization can lead to local immune reactions manifested by infiltration of a large number of inflammatory cells, such as lymphocytes, plasma cells, and macrophages in the treated tissue. In conclusion, the process of PDT-induced tumor necrosis may partly be mediated as an inflammatory response. This is initiated by destruction of endothelial cells (probably also circulating platelets), resulting in hyperemia, edema and thrombosis, and finally in hypoxia or anoxia as well as necrosis of neoplastic cells.

Ways to potentiate the efficiency of photosensitization

The results of PDT in the treatment of hyperproliferative diseases, especially skin, are most encouraging and, by no means, this therapy has the potential of becoming the treatment of choice. Let's review the main advantages of this treatment.

First of all high cosmetic results (minimal dermal damage, little or no scarring) are often superior to conventional treatment methods.

Conventional treatment often leads to mutilation. PDT pretends to be a treatment, which effectively eradicates target tissue without leaving defects and scars.

Besides, a great advantage of many photosensitizing drugs is that they are nontoxic in the dark. Therefore one does not need to worry about the toxicity to liver, bone marrow, kidneys, spleen, etc.

Moreover, there is no significant morbidity, associated with PDT.

Thus, taking into account all stated above advantages it would be an important expansion of the therapist's armamentarium. So far, the priority of method applied to the treating of any type of target tissue might be easily estimated by its long-term follow-up results and cost effectiveness in the competition with traditional modalities. Thus, PDT, as all new modalities, has to prove itself equal or superior to existing therapies and especially cost effectiveness as integral part of it looks as one of serious disadvantages.

In spite of above mentioned doubts concerning PDT,

there are many ways to improve the existing situation and to give an answer to the question whether PDT still can be called “a promising new modality”.

It is well known, that PDT has several side effects, which sometimes cause that the long-term follow-up results are not better than for conventional treatments. Understanding the biological mechanism of PDT action makes a great potential for PDT optimization. Firstly, clinically accepted Photofrin displays prolonged and generalized photosensitivity of the skin. The depth of light penetration of a few millimeters at 630 nm is a limitation for this photosensitizer. Newly developed second-generation photosensitizers generally have absorption peaks at longer wavelengths (far red), faster clearance from normal tissue and more favorable pharmacokinetics, resulting in significantly less skin photosensitivity. Photosensitizers such as Levulan, Foscan, SnET2, Verteporfin, Npe6, Lutrin and Pc4 are under clinical investigation (2, 4, 20). Moreover, every year a large number of new photosensitizers are advanced as potential new photosensitizers in practice. Thus, search for new “candidates” and evaluation of their physico-chemical, photobiological properties is of crucial importance.

Let's look through the possible ways to improve the efficiency of photosensitization and reduce its costs.

Evaluation of new photosensitizers

“...it is one of the most powerful photodynamic agents in nature...” (Walker et al, 1979)

Because of its potential photosensitizing characteristics, numerous investigators have recently focused on hypericin as a novel PDT tool. Hypericin is a hydroxylated phenanthroperylenequinone present in a number of plants of the genus *Hypericum*, widely distributed around the world, most common of which is *Hypericum perforatum* (Fig. 4).

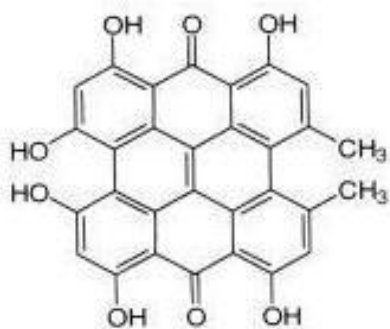


Fig. 4. Chemical structure of hypericin

Reviewing in short the numerous studies on hypericin *in vitro*, it could be summarized that hypericin possesses really powerful phototoxicity on different cell lines (21). Moreover, there is growing body of evidence, suggesting that hypericin exerts in some cases significant antitumor activity, but it is differential and depends on histological origin of tumor (22).

Nevertheless, the results of just two clinical trials with hypericin as a PDT tool have been published. A first clinical study performed at the Boltzmann Institute (Vienna, Austria) used hypericin topically as an effective photochemotherapeutic in recurrent malignant mesothelioma (23). Another clinical study has been performed showing the PDT efficacy of hypericin against basal cell carcinoma and squamous cell carcinoma of the skin (23). Further clinical trials for the treatment of recurrent malignant gliomas are likely in progress at the Department of Neurological Surgery (University of Southern California School of Medicine, LA, USA) (25).

Thus, evaluation of new photosensitizers, comparison of their antitumor activity with well-known, might let us find more effective and wider applicative ones.

Apoptosis as possible key target for increasing efficiency of therapy

After a quarter of century of rapid advances, research has generated complex body of knowledge, revealing hyperproliferative disease, including cancer, to be a result of genome changes. Several lines of evidence indicate that at least six alterations in cell physiology collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis (26). One of them is evasion of apoptosis. It's evident, that knowing of it offers a unique possibility for new oncology target in eradicating tumor cells by restoration of the apoptotic mechanism. More over, combination of PDT – induced apoptosis (mostly from mitochondria) with certain death receptors induced apoptosis (from cell membrane) may be an effective tool to treat hyperproliferating disorders. Consequently, investigation of apoptosis induction after PDT treatment is essential for choosing the best therapeutic approach.

Thus, apoptosis induction in cancer cells may serve for cure. Other interesting approach is to restore the defective apoptotic machinery in the cancer cells, because this is the reason of their resistance to various chemotherapy drugs (27).

Although Thomas et al. transplanted, for the first

time, bone marrow cells into patients in 1957, bone marrow transplantation has not yet reached its full potential (28). A variety of methods have been developed for purging of malignant cells. PDT might be promising in this context. It has been found that merocyanine 540 (MC-540) leads to preferential killing of leukemia cells (Daudi, K-562, Raji and HL-60), whereas 80% of the normal bone marrow cells and 40% of granulocyte macrophage colony forming units were not damaged (29). Furthermore, according to C. H. M. Jamieson (30) leukemia cells take up more benzoporphyrin derivative (BPD) than normal bone marrow cells (acute myeloid leukemia, normal peripheral blood leukocytes, mobilized peripheral blood stem cells).

Thus, the sensitivity of leukemia and lymphoma cells to PDT treatment and especially the possibility to restore the apoptotic machinery in the cells gives a unique opportunity for the effective purging of malignant cells. A number of aspects, involved in the control of apoptotic cell death, might increase the efficiency of PDT by influencing specific signal transduction pathways. For instance, increased protein expression of anti-apoptotic factor Bcl-2 is well recognized in leukemia and melanoma. Besides, Bcl-2 overexpression induces resistance of these cells to chemotherapy and determines a poor clinical outcome.

Moreover, other important proapoptotic factor Bax has been implicated as a major factor in the pathogenesis of leukemia. The expression of Bax relative to that of Bcl-2 alone has, perhaps, a more powerful impact on the control of apoptosis.

Caspases, the main effectors of apoptosis, may be a serious target of cancer therapy. Agents, specifically activating caspases, are able to increase significantly the therapeutic effect. Nuclear factor kappa-B (Nf- κ B) is survival-promoting factor and induces caspase suppression. So, reduction of Nf- κ B expression would be also useful to increase therapeutic efficiency.

Summarizing, investigation of apoptosis induction and signaling might suggest as powerful potential to increase the efficiency of PDT and to potentiate the cure of hematological disease.

Enhancement of photosensitization efficiency by combination with ionizing radiation or hyperthermia

In order to maximize therapeutic outcome and reduce side effects, modern treatment usually is a combination of different modalities.

Actually the efficacy of PDT is also based on the interplay of direct cell killing, vascular damage, in-

flammation, induced hypoxia, etc. Thus, in order to maximize therapeutic outcome and reduce side effects, modern treatment usually is a combination of different modalities. For instance, combination of PDT with ionizing radiation might improve the limited depth of target tissue damage, induced after PDT. Moreover, some additivity is expected in damaging cell key-targets, inactivation of repair systems, induction of apoptosis, etc. Besides, of critical importance would be the determination, if photosensitizers can act as radiosensitizers. If so, the efficiency of combination would be very significant and would allow reduce the costs of treatment very markedly.

During the 1950s and later S. Schwartz and coworkers did extensive studies on the relations of porphyrins and ionizing radiation. About 55 human cancer patients, more than 20,000 mice, many dogs, rabbits and paramecia, more than 100 different porphyrins were included in these investigations. According to S. Schwartz, response to ionizing radiation depends heavily on three factors: porphyrin dose, porphyrin type and tissue type. Moreover, the conclusion was made that the same porphyrin can act as radiosensitizer as well as radioprotector. So far only few very short reports have been published reflecting this problem (30).

Further J. Moan (31) carried out experiments with NHIK 3025 cells. Under aerobic conditions hematoporphyrin (HP) in concentration range 0.5–0.7 mM had no influence on cell growth.

In contrary, K. H. Zhang has showed significant radiosensitization of human malignant glioma cells to ionizing radiation (2–6 Gy) by well-known photosensitizer hypericin. In addition, the efficacy of radiotherapy prior to surgery (40%) for the treatment of maxillofacial tumors has been determined with and without sensitization with hematoporphyrin derivative (HPD) (32). Further H. Kostrom (33) described the interaction of HPD, light and ionizing radiation in rat glioma. ^{60}Co irradiation produced a significant tumor growth inhibition what was increased in the presence of HPD (5–20 mg/kg). It was directly related to the concentration of HPD. Light exposure 30 min prior to ^{60}Co irradiation produced the largest growth inhibition. Moreover D. Y. Chen demonstrated the sensitizing effect of HPD to radiotherapy in the treatment of S180 tumors, transplanted into mice (34). The inhibitory effect on the tumors after radiotherapy alone was 21%, while that after a combination of HPD and radiotherapy was 50%. Z. Lukšienė et al. (35) clearly showed significant radiosensitizing properties of ALA and HPde on two different types of tumors.

Thus, by no means, combination of PDT with ionizing radiation might produce deeper damage of

tumor, but just in the cases, when photosensitizer is able to work as radiosensitizer.

Improvement of photosensitization efficiency by combination with hyperthermia

“Those diseases that medicine does not cure are cured by the knife. Those that the knife does not cure are cured by fire. Those that fire does not cure must be considered incurable”.

Hippocrates

As stated above, tissue oxygenation is one of the most important factors, determining therapeutic outcome after PDT treatment. Growing body of evidence indicates that the absence of oxygen completely inhibits photosensitization. In practice, for instance between 35–40% of cancer patients are anemic and 30% of human carcinomas display areas of hypoxic tissue (36). Thus, tissue hypoxia resulting from a restricted oxygen supply is a common clinical feature and may be decisive for the outcome of PDT treatment. Moreover, if compared, the oxygen concentration required for PDT is ten fold greater, than that found for ionizing radiation. Thus, combining PDT with hyperthermia suggests the possibility to damage hypoxic regions of poor vascularized tumors and remarkably enhance the efficiency of treatment.

Moreover, during PDT the oxygen concentration in the tumor may be further reduced as a result of two processes: (1) oxygen consumption through production of $^1\text{O}_2$ and its irreversible reactions with biomolecules; (2) PDT induced damage to vessels in the tumors leading to a further reduced supply of blood and oxygen to tumor.

Thus, one of the possible ways to enhance tumor cell response to PDT would be combination of the last with hyperthermia (HT). The point is, that PDT treatment results in both direct and indirect tissue damage near the tumor surface, while HT damages tissue both directly and indirectly at the depths where photodynamic effects are minimal or nonexistent (37). The next important feature and disadvantage of PDT is its absolute requirement of molecular oxygen. HT is selectively effective for hypoxic cells, because hypoxic cells with reduced pH are very sensitive to hyperthermic treatment due to insufficient nutrients and poor blood perfusion.

Moreover, the PDT and HT targets in the tumors are similar: PDT induces direct protein inactivation (cell membrane injury), inactivation of repair systems, DNA, induction of apoptosis in tumor cells and indirect (microvasculature) tissue damage, whereas the treat-

ment of HT results in direct cell membrane damage, protein inactivation, injury of repair system, DNA, induction of apoptosis in cells and indirect microvasculature damage in tumors. So HT effect seems in many cases to be able to compensate for the diminished photodynamic effect (38).

In previously described studies various temperatures, sequencing, time interval were given in combination of hyperthermia with PDT. The maximal cell damage *in vitro*, the same as tumor response *in vivo* was reached when treatments followed one another immediately. The question still arises as to whether such improvements in tumor response could also be achieved by delivering the light for PDT in a manner, which would produce thermal as well as photodynamic effect.

So far, there is no data about simultaneous photosensitization and hyperthermic treatment in comparison with sequence-dependent action and it looks like, that this combination might be the most effective (39).

The role of peripheral benzodiazepine receptors in cellular response to photosensitization

A variety of structures are localized on the inner and outer mitochondrial membranes, those could be among the targets for photosensitization by endogenous PpIX. Among these is the mitochondrial (peripheral) benzodiazepine receptor (PBR), the endogenous ligand of which might be protoporphyrin IX.

The PBRs were first described (more than 20 years ago) as BZ binding sites in peripheral (kidney, heart, adrenal) as well as in malignant tissues (40).

The PBR appears to be a heteromeric complex of at least three different subunits, including an isoquinoline binding subunit (18 kDa), a VDAC (32 kDa), and an adenine nucleotide carrier (30 kDa) (Fig. 5) (41).

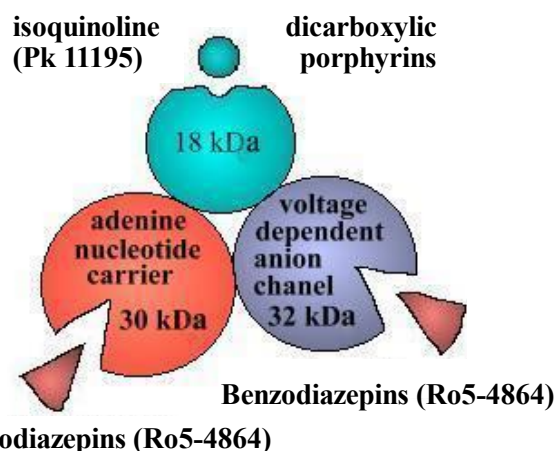


Fig. 5. The structure of peripheral benzodiazepine receptor

Recently, peripheral benzodiazepine receptors (PBR) have attracted attention of many “photodynamists” due to the few reasons. First, during 1980s, data regarding the involvement of the PBRs in cell proliferation have accumulated. Second, some photosensitizers including protoporphyrin and others with nanomolar affinity have been suggested to be endogenous ligands for PBRs (42).

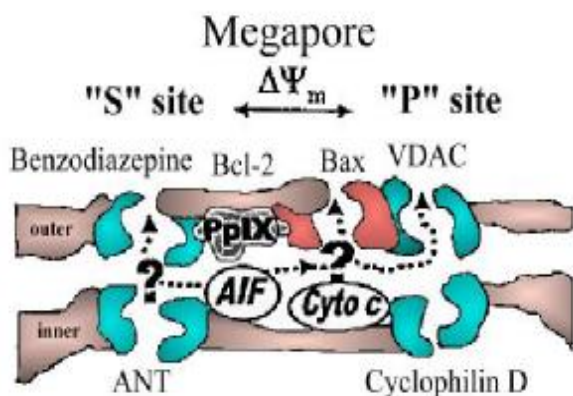


Fig. 6. Formation of megapore in the mitochondrion membrane

The opinion exists that the regulation of cell proliferation by PBR is linked with altered binding characteristics of PBRs, mostly PBRs density and number in neoplastic tissues. Using well-known PBR ligands Ro5-4864 (4'-chlorodiazepam) and PK 11195 (isoquinoline carboxamide derivative) affinity to PBRs in different tissues was determined. In addition, increased PBR density was observed in several tumors. For instance, PBR density in human epithelial ovarian carcinoma was noticed in comparison to benign tumors (5-fold) and normal ovaries (3-fold), 3.2 fold increase in PBR

density for adenocarcinoma of the colon (43). Several studies have demonstrated increased binding site densities for BZ ligands in various brain tumors. In particular, one study showed marked increases in high-grade astrocytoma and glioblastoma cells in comparison with normal brain parenchyma, whereas low-grade gliomas and meningiomas exhibited much lower elevations in PBR binding site densities (44).

In addition, PBRs were found to be highly expressed in aggressive metastatic human breast tumor biopsy samples compared with normal breast tissues (45). Moreover, it was found, that the more aggressive breast cancer cell lines, the more abundant were PBR ligand binding. The study went on to characterize the change in cellular location of PBR protein, when more aggressive and less aggressive breast cancer cell lines were compared. More aggressive cell lines showed a nuclear localization for PBR, as opposed to the “normal” or less aggressive tumor mitochondrial location (45).

A strong and positive correlation has been shown between the affinity of PBR ligands and the antiproliferative activity of mouse thymoma cells (46). Such a correlation was not found for CBR ligands. This finding reinforces the notion of PBR involvement in growth control and cellular proliferation. For instance, M. Pawlikowski et al. (47) noted concentration-dependent inhibition of cellular proliferation in mouse spleen lymphocytes by diazepam and Ro 5-4864.

Moreover, according to our data (48) natural and synthetic ligands might inhibit proliferation of tumor cells and in this content might be used as effective antitumor drugs.

Thus, despite photodynamic therapy is a new and promising cancer treatment modality, potential of its effectiveness and use is not exhausted.

Fotodinaminė terapija: veikimo mechanizmas ir būdai gydymo efektyvumui padidinti

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Raktažodžiai: fotodinaminė terapija, vėžys, fotosensibilizatoriai.

Santrauka. Fotodinaminė terapija – naujas gydymo būdas, kurio pagrindą sudaro fotosensibilizatoriaus suleidimas į veną, jo selektyvus kaupimasis hiperproliferuojančiame audinyje, o po apšvitos matoma šviesa prasideda audinio destrukcija ir žūtis apoptozės arba nekrozės būdu. Metodo veikimo mechanizmas aprašytas nuo šviesos kvantų sugerties audinyje iki to audinio destrukcijos. Nemažai pasiūlymų pateikta fotodinaminės terapijos efektyvumui didinti: pirmiausia įvertinti naujus fotosensibilizatorius, derinti fotosensibilizaciją su kitais gydymo metodais molekuliniam lygmenyje, išnaudojant apoptozės mechanizmą, didinti poveikio efektyvumą derinant su jonizuojančia spinduliuote, hipertermija ir periferinių benzodiazepininių receptorių ligacija.

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