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Review

Photodynamic therapy – mechanisms, photosensitizers and combinations

Stanisław Kwiatkowski[a], Bartosz Knap[b], Dawid Przystupski[a], Jolanta Saczko[c,d], Ewa Kędzierska[b], Karolina Knap-Czop[e], Jolanta Kotlińska[b], Olga Michel[c], Krzysztof Kotowski[a], Julita Kulbacka[c,d,*]

[a] Faculty of Medicine, Wroclaw Medical University, J. Mikulicza-Radeckiego 5, 50-345, Wroclaw, Poland
[b] Chair and Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Chodzki 4a, 20-093, Lublin, Poland
[c] Department of Medical Biochemistry, Wroclaw Medical University, Chalubinskiego 10, 50-368, Wroclaw, Poland
[d] Department of Molecular and Cellular Biology, Wroclaw Medical University, Borowska 211A, 50-556, Wroclaw, Poland
[e] Department of Clinical Genetics, Medical University of Lublin, Radziwillowska 11, 20-080, Lublin, Poland

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ABSTRACT

Photodynamic therapy (PDT) is a modern and non-invasive form of therapy, used in the treatment of non-oncological diseases as well as cancers of various types and locations. It is based on the local or systemic application of a photosensitive compound - the photosensitizer, which is accumulated in pathological tissues. The photosensitizer molecules absorb the light of the appropriate wavelength, initiating the activation processes leading to the selective destruction of the inappropriate cells. The photocytotoxic reactions occur only within the pathological tissues, in the area of photosensitizer distribution, enabling selective destruction. Over the last decade, a significant acceleration in the development of nanotechnology has been observed. The combination of photosensitizers with nanomaterials can improve the photodynamic therapy efficiency and eliminate its side effects as well. The use of nanoparticles enables achievement a targeted method which is focused on specific receptors, and, as a result, increases the selectivity of the photodynamic therapy. The object of this review is the anticancer application of PDT, its advantages and possible modifications to potentiate its effects.

1. Introduction

Photodynamic therapy (PDT) is a modern and non-invasive form of therapy, used in the treatment of non-oncological diseases as well as cancers of various types and locations. Good therapeutic results and the possibility of the parallel application of PDT with other therapeutic protocols make it more commonly used in many fields of medicine [1]. PDT has been successfully used in dermatology, oncology, gynecology and urology.

Photodynamic therapy is based on the local or systemic application of a photosensitive compound - the photosensitizer, which is intensely accumulated in pathological tissues. The photosensitizer molecules absorb light of the appropriate wavelength, initiating the activation processes leading to the selective destruction of the inappropriate cells. Photodynamic therapy is well tolerated by patients because of its selective action. Photodynamic protocols are painless, and the simplicity of their application allows for outpatient use. Photodynamic therapy is also used in the treatment of chronic inflammation and is an interesting alternative in the treatment of drug-resistant bacterial infections [2,3].

The focus of this review is the anticancer application of PDT, its advantages and possible modification to potentiate its effect. Numerous studies indicating the use of photosensitizers in oncology have been carried out over last several decades. Despite the success of PDT, new compounds and innovative methods are still being researched and required to improve the effective use of photodynamic therapy in clinical oncology. Previous studies have led to a significant extension of the possible applications and combinations of photodynamic therapy against cancer cells. In addition to the traditional drug applications, the use of electroporation and nanocarriers is considered to increase the local concentration of the photosensitizer, which results in better efficiency of applied therapy [4].

The goal of the presented work is to summarize the current knowledge on photodynamic therapy. At the beginning, the molecular basis of the photodynamic reaction will be discussed. After discussing the PDT selectivity, a summary of currently used photosensitizers in medicine will be presented. Next, various modifications of photodynamic therapy and possible applications are described.

2. PDT mechanism

Molecular mechanism of photodynamic therapy is based on the three non-toxic components, which produce the desired effects within

* Corresponding author at: Department of Molecular and Cellular Biology, Wroclaw Medical University, Borowska 211A, 50-556, Wroclaw, Poland.
E-mail address: julita.kulbacka@umed.wroc.pl (J. Kulbacka).

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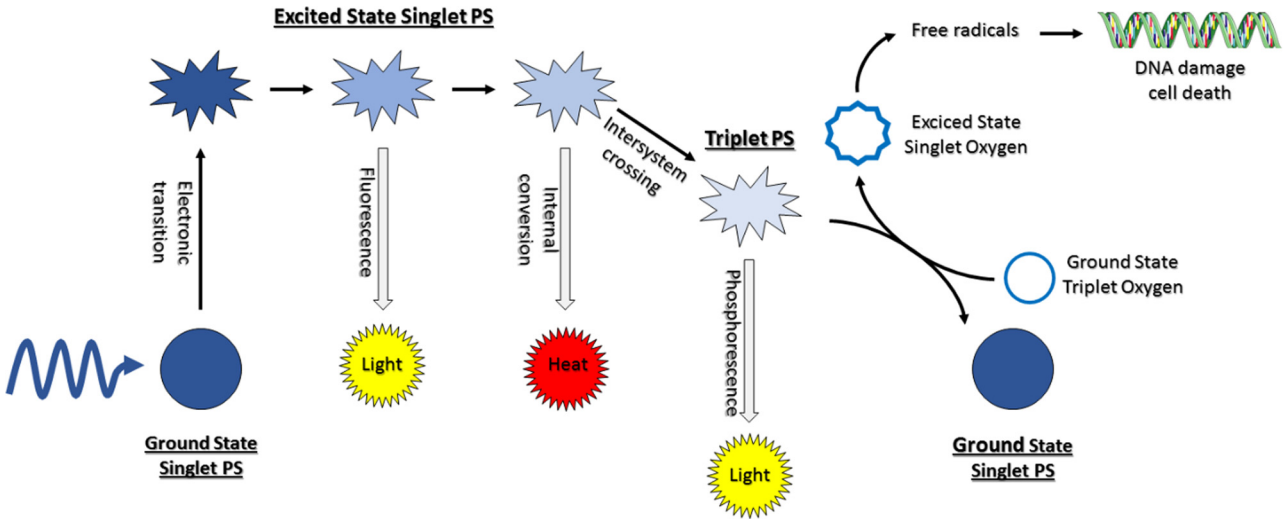


Scheme 1. Mechanism of the photodynamic reaction.
(Modified from [6,7]).

pathological tissues only by mutual interactions between:

- the photosensitizer (PS);
- light with the appropriate wavelength;
- oxygen dissolved in the cells [5].

There are two main mechanisms of the photodynamic reaction. Both are closely dependent on oxygen molecules inside cells. The first stage of both mechanisms is similar. A photosensitizer, after entering the cell, is irradiated with a light wavelength coinciding with the PS absorption spectrum and is converted from the singlet basic energy state S° into the excited singlet state S^1 because of the photon absorption. Part of the energy is radiated in the form of a quantum of fluorescence, and the remaining energy directs a photosensitizer molecule to the excited triplet state T^1 - the proper, therapeutic form of the compound (Scheme 1) [6,7].

2.1. Type I of mechanism of photodynamic reaction

In the excited triplet state T^1, the photosensitizer can transfer energy to the biomolecules from its surroundings. Between the photosensitizer in the T^1 state and the cancerous tissue (substrate), a hydrogen or electron is transferred, which leads to the formation of free radicals and anion radicals of the photosensitizer and the substrate. Electrons interreact with oxygen molecules, which remain in their basic energetic state. This process leads to the production of reactive oxygen species (ROS) - initially in the form of superoxide anion radical ($O_2^{\bullet -}$), which creates further generation of ROS inside the cells. The initiated cascade of reactions leads to the oxidative stress resulting in the destruction of cancer cells [8,9].

2.2. Type II of mechanism of photodynamic reaction

As a result of the photosensitizer's transition into the excited triplet state, energy is transferred directly to the oxygen molecule in the basic energetic state (the basic triplet state). Direct energy transfer between molecules (PS → O_2) is possible because they have the same spins. In this way excited oxygen particles - so-called *singlet oxygen* - are generated, which are characterized by extremely strong oxidizing properties [8,10].

Most organic compounds are in the basic singlet state. However, oxygen molecules are characterized by their triplet state (as the basis) and excitation into the singlet. Owing to this fact, excited photosensitizer particles do not damage organic cell structures and react only with oxygen molecules dissolved in the cytoplasm [11].

It is assumed that mechanism of type II is the most important process conditioning the efficiency of PDT. Nevertheless, the ratio of the contribution of both mechanisms depends on many factors, including: oxygen concentration, tissue dielectric constant and pH and photosensitizer's structure. As the oxygen runs out, the first type of mechanism begins to prevail [7].

Highly reactive oxygen species cause the photodamage of proteins, fats and other molecules in the photosensitized area. This leads to the direct death of tumor cells in the process of apoptosis and / or necrosis [12]. The mutual contribution of different types of cell death depends on the intracellular location of photosensitizer. The damage of mitochondria can lead to apoptosis, cell membrane destructions and loss of integrity can induce necrosis, and damage of lysosomes or endoplasmic reticulum can provoke autophagy [13–15].

2.3. PDT selectivity

The described photocytotoxic reactions occur only within the pathological tissues, in the area of photosensitizer distribution, enabling selective destruction [16]. Photosensitizers accumulate in significantly higher concentrations in cancer cells than in regular cells. The reason of such biodistribution may be the tendency of photosensitizers to combine preferentially with low density lipoproteins (LDL). The role of LDL is to supply tissues with the necessary cholesterol to create membranes during cell division. Vehemently dividing cancer cells show an increased uptake of LDL lipoproteins, which act as a "transporter" of the photosensitizer to the cancerous tissues [17].

In addition, tissues with an increased mitotic activity reveal excessive expression of LDL lipoprotein receptors on the cell surface. The affinity of photosensitizers for serum lipoproteins, in particular for LDL, plays an important role in the delivery of these drugs to the tumor tissue [18,19].

It is known now that PDT leads to a systemic anti-cancer response. Photodynamic therapy affects the vascular system of the tumor and stimulates the immune system. The process of destruction of an inappropriate tissue is complemented by the activation of coagulation processes (occlusion of tumor vessels) and local accumulation of inflammatory cells [20].

Cancer cells that have escaped death by the direct photocytotoxic effects of PDT may still be destroyed via the indirect influence of PDT on tumor blood vessels. Reactive oxygen species damage of vascular

endothelial cells activates clotting processes, aggregate platelets and block vessels by forming thrombi. As a result of vascular occlusion, persistent hypoxia of tumor tissue leads to the cell death [21].

Furthermore, the efficiency of the PDT-method is associated with systemic anti-cancer immune response of the body. PDT destroys the structure of the tumor and thus stimulates direct interaction between immune cells and cancer cells. Direct destruction in tumor tissue leads to the development of a strong inflammatory reaction and neoplasm infiltration by leukocytes. Membrane photodamages lead to the activation of phospholipases, and then cyclooxygenases, causing massive release of inflammatory mediators - lipid hydrolysis products and arachidonic acid metabolites. Photo-injuries of the blood vessel walls attract neutrophils and macrophages. Neutrophil degranulation as well as the release of lysosomal enzymes and chemotactic factors additionally contribute to the destruction of tumor tissue, exacerbating the destruction process initiated by the earlier irradiation [22,23].

3. Photosensitizers

One of the three crucial elements of PDT, apart from light and oxygen, is the presence of photosensitizers. These dyes are defined as substances capable of absorbing light with a specific wavelength, triggering photochemical or photophysical reactions [24]. As in each group of drugs, a set of characteristics and conditions describing the ideal photosensitizer can be distinguished:

- High degree of chemical purity.
- Stability at room temperature.
- Photosensitive effect only in the presence of a specific wavelength.
- High photochemical reactivity; the maximum absorption of light should be at wavelengths from 600 nm to 800 nm. Absorbance of light at a wavelength above 800 nm does not provide enough energy to stimulate oxygen in its state of singlet and production of other reactive oxygen species.
- Absorption minimum in the range from 400 nm to 600 nm. This prevents possible excessive photosensitivity caused by sunlight.
- The absorption bands should not overlap the absorption band of other substances in the body, including endogenous dyes such as melatonin, hemoglobin or oxyhemoglobin.
- Minimal cytotoxicity in the dark.
- Easy solubility in the tissues of the body.
- High selectivity for neoplastic tissues: the photosensitizer should be slowly removed from the affected areas staying there for at least several hours, but be quickly eliminated from healthy tissues, thus minimizing the phototoxic side effects of the therapy.
- Inexpensive and simple synthesis and easy availability [8,24–27].

3.1. 1^{st} generation photosensitizers

The first application of a photosensitizing agent in combination with light can be attributed to a medical student from Munich - Oscar Raab. During experiments with acridine dyes, Raab noticed that fluorescence occurs in protozoa which had been treated with dyes and then irradiated. This phenomenon triggered the consumption of oxygen and the toxic effect, which led to the death of protozoa. Raab presented his observations to Professor Von Tappeiner, who explained and described this phenomenon as a "photodynamic effect" in 1904 [28]. Soon after that, in 1905, the first effective attempt of skin cancer treatment with the use of 5% eosin solution was carried out. However, this therapy did not reach a wider audience and was forgotten for decades [29].

Photosensitizers were introduced to the treatment on a commercial scale for the first time in the 1970s by Dr. Thomas Dougherty and his colleagues [30]. They were testing a water-soluble porphyrin mixture called the "hematoporphyrin derivative" (HpD). HpD was obtained by purification and chemical modification of the first porphyrin used as PS - hematoporphyrin (Hp) (Table 1). HpD compared to Hp showed better tissue selectivity for tumors and less photosensitizing potential on the skin. Subsequently, a mixture of porphyrin dimers and oligomers isolated from HpD was available under the trade name “Photofrin”. Currently, Photofrin - also known as sodium porfimer – has remained the most commonly used PS [31]. Despite wide applications in PDT, the preparation has some limitations of its clinical applications resulting from the following properties: low chemical purity (it is a mixture of over 60 molecules) or poor tissue penetration due to maximum absorption at a relatively short wavelength - 630 nm. In addition, after PDT, skin hypersensitivity to light for several weeks because of long half-life of PS and its high accumulation in the skin occurs. The disadvantages of the first generation photosensitizers forced the need of investigating new compounds and initiated the development of the second generation photosensitizers [32,33].

3.2. 2^{nd} generation photosensitizers

As early as in the 1980s, studies on the next generation of photosensitizers began. Several hundred substances with potential photosensitizing properties had been proposed, only a few of which were used in clinical trials. The number of substances officially approved for clinical use in anti-cancer PDT is even smaller.

Currently, the group of the second generation photosensitizers includes hematoporphyrin derivatives and synthetic photosensitizers such as: 5-aminolevulinic acid, benzoporphyrin derivatives, texaphyrins, thiopurine derivatives, chlorin [34,35] as well as bacteriochlorin analogues and phthalocyanines (Table 1) [34]. The use of 5-aminolevulinic acid (ALA) (Table 1) turned out to be an important discovery - the precursor of protoporphyrin IX. ALA is a kind of prodrug that becomes an active PS only after being transformed into the protoporhyrin. For that reason, ALA or its esters can be used topically or orally in many clinical applications [36,37].

The second-generation photosensitizers are characterized by a higher chemical purity, higher yield of singlet oxygen formation and better penetration to deeply located tissues due to their maximum absorption in the wavelength range 650–800 nm. In addition, they demonstrate fewer side effects, which results from a higher selectivity for cancerous tissues and faster elimination of the photosensitizer from body. The main disadvantage of the second generation PS is their poor solubility in water, which is a significantly limiting factor in their intravenous administration and forces the search for new methods of drug delivery [8].

3.3. 3^{rd} generation photosensitizers

The development of the third generation photosensitizers is based on the synthesis of substances with higher affinity to the tumor tissue, which reduces damage to surrounding, healthy tissues. The problem for the widespread clinical application of photodynamic therapy in oncology is also the difficulty with the preparation of a pharmaceutical procedure that would enable the parenteral administration of photosensitizers. New drug delivery systems are emerging that effectively increase the bioavailability of the photodynamic method [38].

In order to increase the selectivity of the drug, the following modifications of photodynamic therapy are used:

- combinations of second generation photosensitizers with molecules focused on the target receptor,
- combinations of photosensitizers with LDL lipoprotein, due to the fact that the proliferating tumor cells need more cholesterol for the synthesis of cell walls,
- conjugation of a photosensitizer with a monoclonal antibody directed to the specific antigen of cancer cell,
- the use of tumor surface markers such as growth factor receptors, transferrin receptors or hormones (e.g., insulin).

Table 1
Photosensitizer families and their representatives.
(Based on [18,19,24,25,28,31,34,35]).

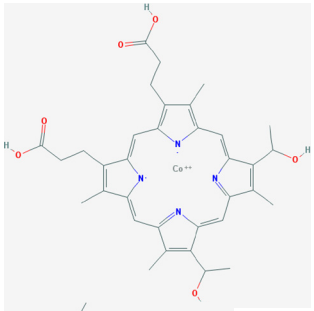
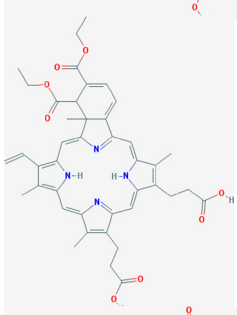
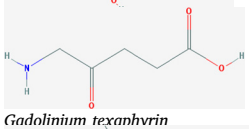
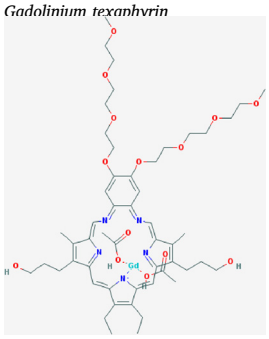
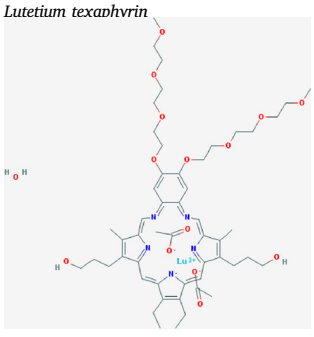
Porphyrin family	*HpD (hematoporphyrin derivative)*	
	BPD (*benzoporphyrin derivative*)	
	ALA (*5-aminolevulinic acid*)	
	Texaphyrins	*Gadolinium texaphyrin*  *Lutetium texaphyrin* 

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Table 1 (*continued*)

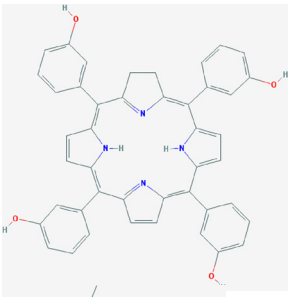
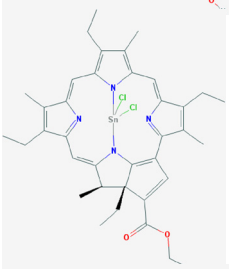
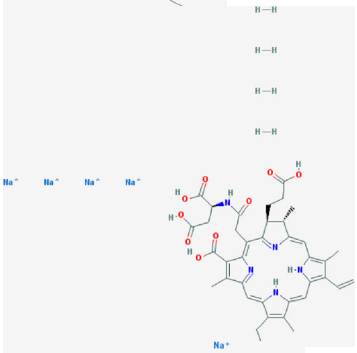
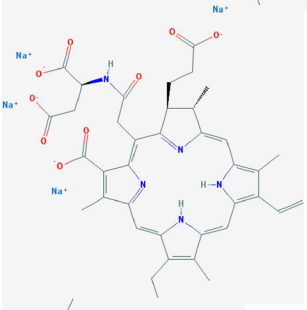
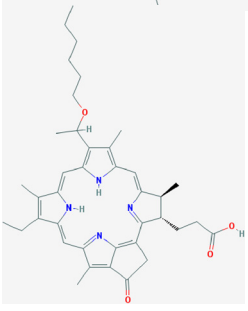
Chlorin family	Temoporfin	
	Purlytin (*tin-ethyl-etiopurpurin*)	
	NPe6 (*mono-L-aspartyl chlorin e6*)	
	LS11 (*Talaporfin sodium*)	
	HPPH (*Photochlor*)	

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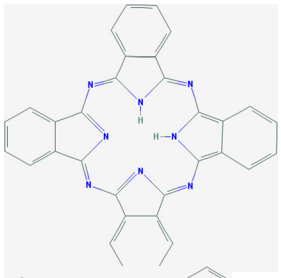
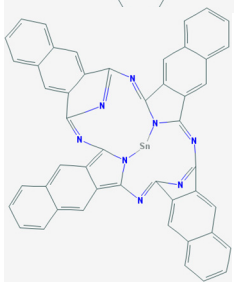
Table 1 (*continued*)

Dyes family	Phthalocyanine	
	Naphthalocyanine (*tin 2,3-naphthalocyanine*)	

These solutions allow for an enhancement of the the selectivity and for a greater accumulation of the photosensitizer in affected areas, and thus - give the the possibility to reduce doses of the drug while maintaining satisfactory therapeutic effects [39,40].

4. PDT modifications

4.1. Nanotechnology on PDT

Over the last decade, a significant acceleration in the development of nanotechnology has been observed. The so-called *nanomedicine* uses nanomaterial platforms for diagnostic and therapeutic procedures, enabling the precise drug delivery to target tissues and improving the effectiveness of anti-cancer therapy [41–43].

The combination of photosensitizers with nanomaterials can improve the photodynamic therapy efficiency and also eliminate its side effects. The use of nanoparticles makes it possible to achieve a targeted method which is focused on specific receptors, and as a result, increases the selectivity of photodynamic therapy. Photosensitizers may be encapsulated in or immobilized to nanoplatforms by covalent and non-covalent interactions [44].

Most photosensitizers are highly hydrophobic substances which aggregate in an aqueous environment. The aggregation process reduces the efficiency of photodynamic therapy. Photosensitizers have to remain in a monomeric form to be photoactive [45]. Maintaining this configuration is possible due to conjugation of photosensitizers with nanoparticles. The bioavailability of hydrophobic porphyrins is effectively increased by forming covalent connections to hydrophilic polymer molecules [25]. The use of polymeric nanoparticles e.g. micelles in PDT allows for the targeted delivery of more photosensitizer molecules to the tumor region and prevents degradation of the photosensitizer before reaching the target tumor tissue [46]. In addition, the use of polymers allows the simultaneous attachment of further ligands to the PS molecules, for example contrast substances or fluorescent markers enabling clinical image explorations [47,48].

Research of meso-tetra-4-hydroxyphenylporphyrin molecules (mTHPP), combined with a polyethylene glycol molecule (PEG) with different numbers of “mers”, has showed that the formed complex improved the solubility of porphyrin residues and reduced their aggregation in the aquatic environment [49,50]. Increasing the hydrophilic properties of the photosensitizer results in the improved selectivity and higher efficacy of the photodynamic therapy. Zeisser-Labouebe studies support the use of another polymer - poly(lactic-co-glycolic acid) (PLGA) in combination with the hypericin photosensitizer in the treatment of Nu-Tu19 ovarian cancer cells *in vitro* [51]. Polyacrylamide (PAA) is another polymer that prevents aggregation of photosensitizers. PAA is a non-toxic substance which can be administered systemically due to the highly-water soluble properties [52]. Many papers confirm the increase in the efficacy of PDT after using a photosensitizer-PAA nanoplatform. Polyacrylamide can be used with photosensitizers: methylene blue (MB) [53,54] and porphyrins [55]. An increase in PDT efficiency with the use of PAA in the treatment of C6 glioma cells was noticed [56]. Other polymer with proven efficacy was loaded by N-(2-hydroxypropyl)methacrylamide (HPMA) and effectively used in PDT protocol in the treatment of neuroblastoma and human ovarian carcinoma *in vitro* [57].

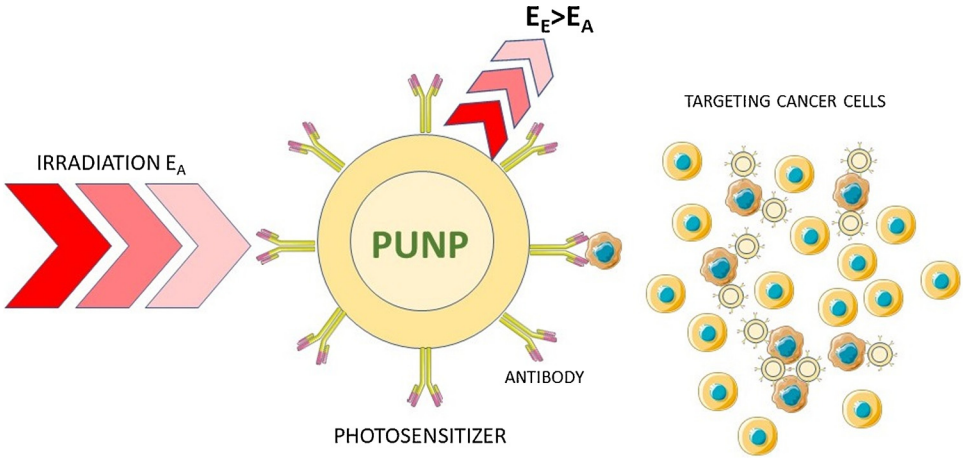
Inorganic substances can be used to create photosensitive conjugates. A promising way to increase the selectivity of PDT is to use gold nanoparticles as a drug delivery system [58,59]. Furthermore, the photosensitizer constructs with extremely stable silicon nanoparticles which are well absorbed by tumor cells have been found [60]. They are degraded in a human body to the easily eliminated silicic acid.

The latest knowledge indicates that nanocarriers modification and technology of synthesis can significantly enhance drug delivery [41,44,50]. Komiyama et al. also proposed a functionalization of single DNAs to achieve stronger DNA binding, DNA aptamers and DNAzymes. It means that we are able to develop intelligent systems which are programmable assemblies of DNAs (so called DNA Origami) and efficiently use them for smart drug delivery [61].

Photosensitizers of the next generation are also PUNP type photosensitizers (*Photon Upconverting Nanoparticles*). They are made of photosensitive compounds and nanoparticles which core has the ability to convert energy obtained from photons. The uniqueness of the system lies in the fact that the radiation emitted by the core has higher energy than the absorbed photon. In addition, the core can absorb infrared radiation, which penetrates tissue to a depth even several times greater than visible radiation. This system accumulates in a tumor tissue in a highly selective manner due to labeling with antibodies (Scheme 2) [62,63].

4.2. Application of liposomes and lipoproteins

The usage of liposomes may be a promising system of the photosensitizer’s targeted delivery to the tumor tissue. Lipid vesicles composed of one or more concentrically arranged phospholipid bilayers are currently the subject of investigation. Experimental and clinical studies



Scheme 2. Mechanism of PUNPs' action in photodynamic reaction (E_A – energy of absorption, E_E – emitted energy). (Modified from [63]).

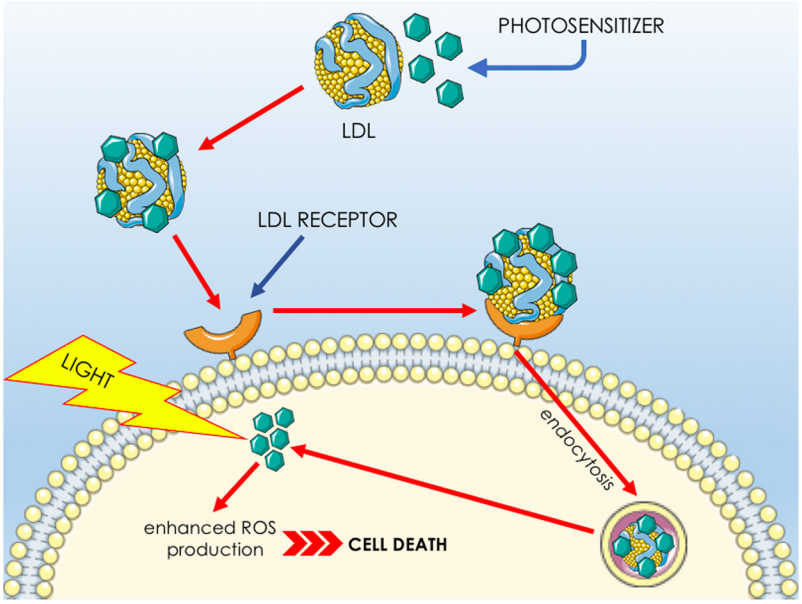
have confirmed the role of liposome carriers as effective photosensitive delivery systems in PDT. Selective accumulation of these nanocarriers occurs due to the increased vascular permeability and retention of various molecules in tumor tissue. A leaky network of blood vessels leads to the formation of an abnormal endothelial barrier and increased vascular permeability within the tumor. This gives an opportunity to reach the target tissue by a simple diffusion. The first photosensitizer loaded in the liposome was HpD. Studies by Cozzani et al. on the HeLa cell line have shown a much stronger photodynamic effect after therapy using liposome constructs containing HpD as an active cargo [64]. Furthermore, the liposomal benzoporphyrin derivative monoacid (BPD-MA) was approved for treatment in Switzerland and the USA [65,66].

Due to the presence of a large number of receptors for low-density lipoproteins (LDL) on the surface of tumor cells, it is also beneficial to combine photosensitizers with LDL [67,68]. Lipoproteins play an important role in the transport and release of photosensitizer molecules to cancer cells. Several studies show that a photosensitizer bonded non-covalently to LDL prior to administration leads to an increase in PDT efficiency compared to the administration of the photosensitizer itself (Scheme 3) [63,69,70].

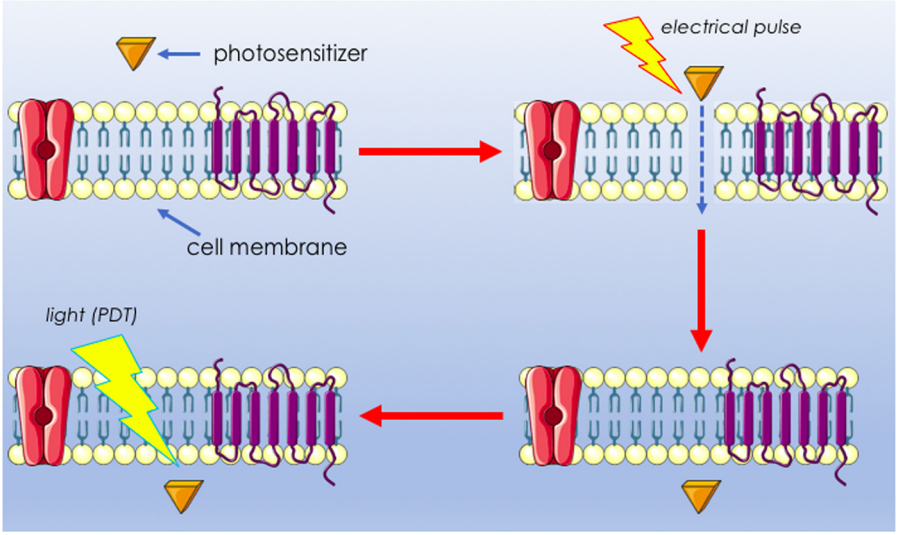
4.3. PDT supported by electroporation

Electroporation (EP) is a technique of reversible or irreversible cell membrane unsealing induced by electrical pulses. The reversible EP variant is effectively applied for enhancement of cell membrane permeability to achieve easier transport of drugs or to enable gene transfection [71,72]. Under the influence of a strong electric field, the lipids in the cell membrane change their structure and undergo reorganization. Transient hydrophilic “pores” are formed, constituting an additional route for the transport of molecules through the cell membrane [73]. The method based on the use of electroporation for the application of cytostatics is called electrochemotherapy (ECT) [74]. Due to the temporal destabilization of cell membranes by the pulsed electric field, the concentration of cytostatic achieved in cancer tissue is higher and chemotherapy is complicated with less severe adverse effects [71].

Electroporation can be used as an effective method to increase the transport of the photosensitizer to the interior of pathological cells (Scheme 4). Labanauskiene et al. showed that the combination of electroporation and photodynamic therapy using chlorine e6 and AlPcS4 phthalocyanine as photosensitizers results in an increase in



Scheme 3. Mechanism of LDL-nanocarriers photosensitizer's delivery. (Based on [70]).



Scheme 4. PDT supported by electroporation.
(Based on [83]).

cytotoxicity after the EP-PDT procedure. The research was carried out on the line of Chinese hamster lung fibroblast cells DC-3 F [75]. Furthermore, studies by Kulbacka et al. demonstrated that the combination of EP with PDT contributed to a significant reduction in the time of photosensitizer incubation with mammary gland adenocarcinoma cells as well as, and provided a simultaneous 10-fold increase in the efficiency of the PDT's efficacy [76]. The other studies combined also electroporation phenomena with clinically approved Photofrin [77–82].

5. Conclusions

Photodynamic therapy is based on the possibility of the selective destruction of pathological tissues with accumulated photosensitizer. Comparing to other therapeutic methods in oncology, PDT is distinguished by its selectivity with equivalent therapeutic results. The growing interest in the treatment of tumors with optical techniques results from their non-invasiveness and high sensitivity. In addition, the new methods increasing the effectiveness of PDT are constantly being discovered. Combinations of the method with other techniques allow for a significant improvement in the treatment results and reduce the side effects of the therapy. Considering the above aspects, photodynamic therapy is gaining more and more prominent position - not only among clinicians, but especially among patients. Rapid development and advances in technology and optoelectronic equipment can favor the implementation of the photodynamic method in clinical practice.

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