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Current Knowledge of Photodynamic Therapy

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Abstract: Photodynamic Therapy (PDT) is an innovative treatment method that uses the synergistic effect of light, photosensitizing drugs, and reactive oxygen species to destroy cancer cells selectively. This advanced technology has the potential to revolutionize the field of medicine through its versatile application in various areas. In addition to the scientific aspects related to PDT, social and general issues are worth discussing to highlight the importance of this therapy for society as a whole. The potential to reduce healthcare costs associated with PDT is also an important social consideration. The use of this therapy may lead to shorter hospital stays and avoidance of costly surgical procedures, which may benefit both patients and healthcare systems. Moreover, thanks to its versatility, PDT can be used in various fields of medicine, which opens the way to treating various diseases, not only cancer.

Keywords: photodynamic therapy; photosensitizer; nanotechnology; quantum dots.

1. Introduction

The scientific and social issues are an introduction to the full understanding of the potential and importance of PDT in medicine [1-10]. In oncology, PDT has shown promising results as a treatment method for various skin cancers [6]. Photosensitizing drugs, selectively absorbed by cancer cells and then activated with light of an appropriate wavelength, enable targeted destruction of these cells [7]. In society, we are increasingly faced with challenges related to cancer, which affects millions of people around the world [3]. PDT, as a promising therapy, has the potential to change the way we counteract these diseases. One of the key aspects of PDT is its minimal invasiveness, which means that it can be an attractive alternative to traditional treatment methods, which are often associated with serious side effects [4]. In addition, PDT can be used topically, enabling precise destruction of changed cells, which minimizes damage to healthy tissue.

Innovative research also focuses on further optimization of photosensitizing drugs and light delivery techniques [8]. Introducing nanotechnology, including photosensitizing nanoparticles, can significantly improve the selectivity and bioavailability of PDT therapy [9]. Additionally, developing new light sources with appropriate properties may increase the depth of light penetration and enable effective treatment of cancer lesions deep into the tissues.

The rest of the review article will discuss the latest research, technological advances, and challenges related to PDT to provide the reader with comprehensive knowledge on the subject and to show the prospects for further development of this fascinating field standing on the border of the sciences of medicine [11].

2. Differences Between PDT and Traditional Cancer Treatments

An ideal cancer therapy would not only destroy the primary tumor. Still, it would also stimulate the immune system to recognize, track down, and destroy any remaining cancer cells, whether they are located at or near the site of the primary tumor or in the case of distant micrometastases.

Unlike surgery, radiotherapy, and chemotherapy, which are mainly immunosuppressive, PDT causes acute inflammation. Chemotherapy is toxic to the bone marrow. Low doses of ionizing radiation [12] or chemotherapy [13] may have an immunostimulating effect. [14]. However, PDT, like other local anticancer therapies such as cryotherapy [15] and hyperthermia [16], may induce acute inflammation in the treatment area. It is this inflammation that can contribute to systemic activation of the immune system. As a result of PDT, various cytokines and other inflammatory signals are released, which attract leukocytes, mainly neutrophils and macrophages, to the tumor area, and the expression of tumor cell antigens increases [17].

3. Mechanism of Action of PDT in the Destruction of Cancer Cells

Photodynamic Therapy (PDT) is an innovative treatment method that uses the synergistic effect of light, photosensitizing drugs, and reactive oxygen species to destroy cancer cells [18] selectively. The mechanism of action of PDT is based on the basic principles of photochemistry and photophysics, in which photosensitizers - compounds activated by light of an appropriate wavelength - are initially introduced into the body [19]. When photosensitizers accumulate in cancer tissues, irradiating them with appropriate light leads to generating reactive oxygen species that damage and destroy cancer cells [1].

Pathological cell death may occur as a result of necrosis and/or apoptosis. PDT also affects tumor vasculature, whereby irradiation and ROS production cause vessel occlusion, restricting access to oxygen and nutrients. Also important is the effect of PDT on the immune system, which may be immunostimulatory or immunosuppressive [20].

3.1. Stages of action of PDT in the destruction of cancer cells.

3.1.1. Administration of a photosensitizing substance.

The first step in photodynamic therapy is to administer a photosensitizing substance to the patient, which has the ability to accumulate in cancer cells [11]. The photosensitizer, usually administered intravenously or topically, is selectively absorbed by cancer cells due to their changed structure and rapid division. This allows the photosensitizing substance to concentrate in areas of the tumor preferentially.

3.1.2. Light activation.

After the photosensitizing substance accumulates in cancer cells, the target area is irradiated with light of a specific wavelength [20]. The photosensitizer absorbs photons (hv)

and enters a state of short-term excitation (reactive oxygen species (ROS), such as singlet oxygen - 1PS *). 1PS* can lose energy through internal conversion to heat or by emission of light (fluorescence). They can also transform into the more stable triplet state 3PS* thanks to an intersystem transition [21], in which the excited electron changes its spin and produces a longer-lived triplet state [20]. PS* can return to the singlet state 1PS (via fluorescence), and two types of reactions with neighboring molecules can be activated.

TYPE I involves the transfer of an electron or proton through 3PS* and forming organic radicals. These radicals can interact with cellular oxygen, leading to the formation of cytotoxic reactive oxygen species (ROS) (e.g., superoxide anion (O_2–*)), hydroperoxide radical (HOO*), peroxides (H_2O_2, ROOH) and hydroxyl radical (HO*); this initiates chain reactions of free radicals.

TYPE II initiates energy transfer between the excited triplet states of 3PS* and molecular oxygen. Triplet oxygen ($3O_2$) is formed, which produces singlet oxygen, which is a strong oxidant.

Type I and type II photochemical reactions can occur simultaneously, and their ratio depends mainly on the photochemical and photophysical properties of PS and the concentrations of substrate and cellular oxygen [21]. A type I reaction often leads to more severe damage than a type II reaction. In type I PDT, PSs are consumed and must be regenerated [22]. Conventional type II PDT has great anticancer potential, but hypoxia seriously hampers its effectiveness [23].

3.1.3. Cell damage.

Reactive oxygen species generated during the photosensitizer activation lead to damage to various cellular structures [24]. Figure 1 shows the mechanism of PDT.

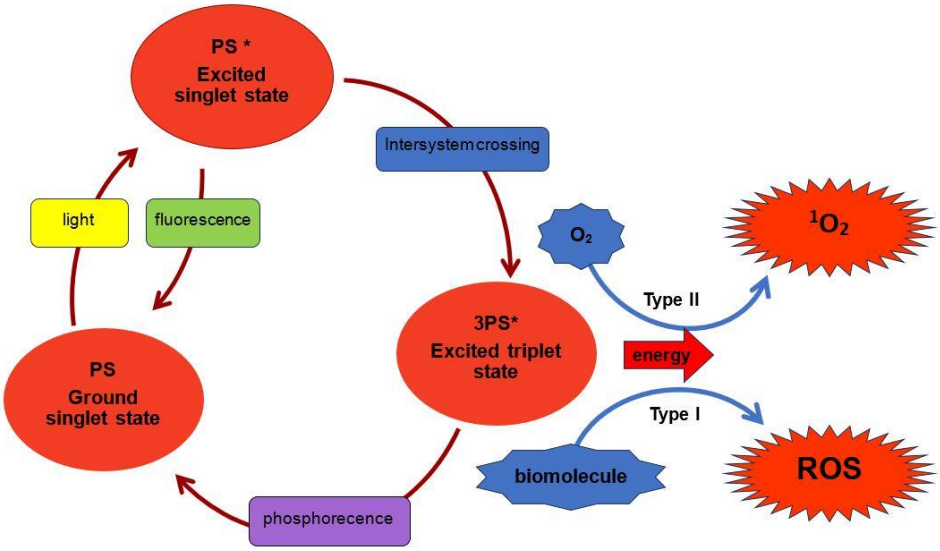

Figure 1. Mechanism of PDT.

The controlled distribution of the photosensitizer in various organelles and cellular structures is important in activating various types of pathways leading to cell death.

Photosensitizers often concentrate in the cell membrane, Golgi apparatus, lysosomes, endoplasmic reticulum, and mitochondria, resulting in cell destruction. The release of singlet oxygen by photodynamic therapy at the cell membrane causes cell death via the HOCl signaling pathway or by disintegrating the cell membrane, which generates cell swelling and rupture. When singlet oxygen is produced in the Golgi apparatus, it results in cell death,

dependent on mitochondrial function. Disruption of lysosomes by singlet oxygen leads to increased permeability of the outer membrane of mitochondria, while photodynamic activity in the endoplasmic reticulum causes abnormal formation of protein structure, which triggers the protein unfolding response (URP) and, consequently, activation of caspase-3 [25].

As a result, photodynamic therapy uses precise targeting of photo-sensitizers at various cellular locations, which diversely leads to cancer cell death.

3.1.4. Cell death.

Cell damage caused by reactive oxygen species triggers various cell death mechanisms [26]. One of the key mechanisms is apoptosis, a controlled form of cell death. The mechanism of apoptosis is very complex and involves many signaling molecules whose action causes specific molecular changes. Research to date suggests that there are two main apoptotic pathways: the extrinsic (death receptor) pathway and the intrinsic (mitochondrial) pathway.

Extrinsic pathway Oxidative stress caused by reactive oxygen species (ROS) generated by photodynamic therapy (PDT) causes activation of the Fas receptor through its oligomerization. Fas binds to the adapter protein FADD and then to procaspase-8, forming a complex. This complex is called the death-inducing signaling complex (DISC), which autocatalyzes by activating procaspase-8 to caspase-8. Caspase-8 cleaves caspase-3 and caspase-7 [24]. Caspase-3 catalyzes the cleavage of many important cellular proteins and is the most frequently activated death protease [27]. Caspase-3 releases caspase-activated deoxyribonuclease (CAD) from the caspase-activated DNase (ICAD) inhibitor, which induces DNA fragmentation. Caspase-7 degrades cellular proteins.[25] CAD activation also induces the degradation of internucleosomal DNA during apoptosis [28,29].

The intrinsic pathway link between the correlation of singlet oxygen occurrence and apoptosis via mitochondria is not fully understood. Singlet oxygen can activate mitochondrial membrane permeability change (MPT) [30]. In 1999, it was confirmed that the effect of PDT products focuses on the Bcl-2 protein [31]. Proteins from the Bcl-2 family are responsible for controlling the permeability of the mitochondrial membrane. Bax is a pro-apoptotic protein from the Bcl-2 family and increases mitochondrial membrane permeability, while Bcl-2 stabilizes and reduces membrane permeability. Bcl-2 binds to pro-apoptotic proteins (Bax, Bak, Bid), preventing their aggregation and thus maintaining the integrity of the mitochondrial membrane [32]. Due to their opposing roles, the concentration of Bax and Blc-2 proteins further determines the cell's fate. When a cancer cell is exposed to PDT, Bcl-2 proteins are destroyed, which prevents interaction with Bax, which drastically increases the permeability of the outer mitochondrial membrane, thus releasing into the cytoplasm: ALF, endonuclease G, CAD, cytochrome c, and the second mitochondrial caspase activator (SMAC) [33]. Also, PDT's damage to the lysosomal membrane results in the release of cathepsin D and B into the cytoplasm. Bid is proteolytically activated, which increases the permeability of the outer mitochondrial membrane [34].

The released cytochrome C binds to apoptotic protease-activating factor 1 (APAF1), which leads to its conformational change. This allows APAF-1 to form multimers called apoptosomes, which provide a platform for activating caspases. The apoptosome then binds and activates procaspase-9 to caspase-9 [35]. Caspase-9 activates caspase-3 and caspase-7, leading to apoptosis [36].

SMAC has a different action compared to cytochrome c released into the cytoplasm. Its main function is to antagonize the activity of apoptosis inhibitors associated with proteins

(IAPs), which indirectly contribute to the activation of the caspase cascade and the induction of apoptosis [37,38]. In contrast, the released AIF acts in the cell nucleus, where it causes DNA fragmentation and nuclear chromatin condensation [39].

3.1.5. Cell degradation.

Activated caspases, especially caspase-3 and caspase-7, lead to proteolytic degradation of proteins crucial for maintaining cellular structure and function [35,38].

Furthermore, other death mechanisms, such as necrosis, an autophagy-related cell death, may also participate in the elimination of cancer cells.

There is a relationship between the type of cancer cell death and their ability to induce an immune response. Both apoptosis and necrosis can trigger immune responses, although the mechanisms may differ. [20]

The balance between apoptosis and necrosis that occurs after a tumor cell is subjected to PDT *in vitro* depends on several parameters, including the total PDT dose (PDT dose is the product of PS concentration and light), intracellular location of PS, oxygen concentration, and cell type [40].

3.1.6. Immune response.

In addition to direct damage to tumor cells, PDT can stimulate the body's immune response [1].

PDT induces both apoptotic and necrotic cell death. These cells are phagocytosed by dendritic cells (DCs). DCs mature after stimulation by cytokines released at the inflammation site. DCs present antigens to T lymphocytes, which become activated, become effector T cells, and, attracted by chemokines, migrate to the tumor and kill tumor cells [20].

Damaging cancer cells and releasing their fragments also activates the immune system, which can recognize and fight remaining cancer cells. This phenomenon is called the immunostimulatory effect of PDT. Singlet oxygen produced by photodynamic therapy has been found to inhibit catalase activity, so in the absence of catalase activity, hydrogen peroxide accumulates, which triggers peroxidase (POD) to initiate HOCl signaling. HOCl signaling causes immunogenic modulation that triggers the immune system to eliminate cancer cells [41].

As a result, the mechanism of action of PDT in the destruction of cancer cells is based on several mechanisms that, working together, can result in good effectiveness of this therapy. This innovative therapeutic method offers the potential to effectively and selectively combat cancer cells while minimizing damage to healthy surrounding tissues.

4. Photosensitizers – Three Generations

Photosensitizers that are used in PDT can be divided into three generations based on their evolution.

The first generation of photosensitizers includes naturally occurring porphyrins and their derivatives (hematoporphyrins (HpD) and photophrin II). The first-generation photosensitizer contains somewhat complex ingredients, which negatively impact tissue selectivity and the stability of photodynamic damage intensity. The effectiveness they achieved was limited by the wavelength emitted by infrared (approximately 630 nm), which does not penetrate lesions located in the deeper layers of tissues and stops at the surface structures of the body [34], which may result in photosensitive cutaneous toxicity [42].

Sodium porfimer or Photofrin also belongs to this class and has been approved for clinical PDT treatment of several precancerous lesions and malignancies in the US. The current clinical formulation does not involve drug combination with a nanoparticle carrier; however, several promising preclinical studies have been conducted using nanoformulations of porphyrin photosensitizers. For example, Chen et al. used human serum albumin nanoparticles to deliver 5, 10, 15, 20-tetrakis(m-hydroxyphenyl)porphyrin (mTHPP, a porphyrin derivative) and pheophorbides (chlorine derivatives) to leukemic cells [43–45]. It was found that the nanoparticles were absorbed by endocytosis and the lysosomal mechanism, leading to approximately 50% of cell death due to apoptosis [44].

Second-generation sensitizers are synthetic compounds that are mostly based on an appropriately modified porphyrin structure and include: benzoporphyrins, purpurins, texaphyrins, phthalocyanines, naphthalocyanines and protoporphyrin IX (PpIX). Second-generation photosensitizers may also have a structure based on chlorine structures. This group includes monoaspartyl chlorine e6 (NPe6), temoporfin, and hexylpyropheophorbide (HPPH) [34].

Studies have shown that PpIX had a longer absorption wavelength in erythroleukemia cells and that protoporphyrin IX is a heme precursor involved in heme metabolism by connecting mitochondrial transport proteins. Another commonly used photosensitizer is 5-aminolevulinic acid (ALA), the biological precursor of PpIX [46].

Compared to the first-generation, second-generation photosensitizers are characterized by higher purity, photosensitivity, tissue selectivity, and longer absorption wavelength in the NIR visible range (650-800 nm) [47]. However, they were still characterized by poor solubility in water, short time of presence in the body, and low selectivity towards cancer [48].

A breakthrough moment was the creation of new second-generation photosensitizers with a modified chemical structure of the original photosensitizer, providing properties that enable the targeting of specific cell organelles and thus increase the anticancer effect. Examples of such compounds include targeting mitochondria with DLC (delocalized lipophilic cations), which can preferentially localize to mitochondria [49]. Based on the DLC structure, three DLC-porphyrin conjugates were created: a modified porphyrin-rhodamine B cation core, a modified porphyrin-mono-triphenylphosphonium cation core, and a modified porphyrin-di-tPP cation core [50]. Most new porphyrins are excited by longer wavelength light, so deeper light penetration by the photosensitizer is required for further studies [51].

In recent years, scientists have developed third-generation photosensitizers to address the problems encountered by previous generations. Using chemical modifications, nanosystems for antibody delivery or conjugation [52].

Chemical modifications aimed at improving targeting capabilities led to the discovery of new-generation photosensitizers, such as mTHPC, introduced by Berenbaum [53]. There are some doubts about whether the drug is a second or third-generation photosensitizer [54]. Prospects for developing the third generation of photosensitizers are based on minimizing phototoxicity in healthy tissues such as skin [52]. Considerable efforts have been made to develop specific vehicles to deliver photosensitizers [53].

One of the third-generation photosensitizers is chlorin E6 (Ce6), an FDA-approved photosensitizer with desirable clinical properties for photodynamic therapy. Ce6 has a high capacity to produce reactive oxygen species (ROS) and has anticancer effects against many types of cancer [54]. Other factors that predispose Ce6 to use in PDT are its strong NIR absorption (in the near-infrared range 650–800 nm) and the ability to be embedded in gold

vesicles (GVs) [55]. Hydrophobicity is the main disadvantage of Ce6, which leads to its poor biodistribution and rapid removal from circulation [54]. To overcome this drawback, researchers designed and manufactured several nanosystems with which Ce6 was combined by forming ionic complexes to enhance tumor uptake and improve ROS production [56]. Thanks to the modification of Ce6 with a nanosystem, chlorin E6 has become a promising third-generation photosensitizer [54].

Recently, an innovative type of green titanium (G-TiO_2-x) was created, which has a unique ability to absorb near-infrared (NIR), especially around 920 nm - this feature allows for improved tissue penetration depth [57]. What's even more promising is that G-TiO_2-x is designed to target mitochondria specifically. Combination with the triphenylphosphonium ligand (TPP) allowed for targeted PDT/PTT therapy on mitochondria, which are the preferred organelles in cancer therapy [58]. PDT/PTT therapy using green titanium has achieved excellent results both *in vitro* and *in vivo*. Importantly, this was achieved at relatively low laser power (980 nm, 0.72 W/cm^2) and low material dosage, which emphasizes the safety of this therapy. Importantly, this material has low toxicity, which suggests its high biocompatibility [57]. This novel G-TiO_2-x opens new perspectives for future precise and minimally invasive cancer treatment.

5. Conclusions

When creating nanoparticles for PDT, scientists ask themselves certain questions and assumptions regarding whether the photosensitizer should be enclosed in the nanoparticle or covalently bound to it. This answer gives rise to further questions directed and conditioned by this answer. If the photosensitizer is non-covalently closed, it is likely to be released more easily and, therefore, better absorbed by the cells. However, there is a danger and risk that it may be released prematurely, preventing it from accumulating adequately at its final destination. An ideal cancer therapy should not only destroy the primary tumor but also stimulate the immune system to recognize, track, and destroy any remaining cancer cells, regardless of where they are located. Photodynamic Therapy (PDT) is an innovative treatment method that uses the synergistic effect of light, photosensitizing drugs, and reactive oxygen species to destroy cancer cells selectively. As a result, the mechanism of action of PDT in the destruction of cancer cells is based on several mechanisms that, working together, may result in good effectiveness of this therapy. This innovative therapeutic method offers the potential to effectively and selectively combat cancer cells while minimizing damage to healthy surrounding tissues. There are many other nanoparticles with wide applications in biomedicine, the development of which may be crucial, making them useful systems providing photosensitizers for photodynamic therapy.

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Conflicts of Interest

The authors declare no conflicts of interest.

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