Photodynamic Therapy Curbing the Uncontrolled Proliferations: An Orodental Perspective

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ABSTRACT

Cancer is a terminology known worldwide for a variety of diseases that have in common the proliferation of specific cell populations, with potential to invade nearby and/or distant tissues. Oral cancer is the 11th most common cancer in the world. Traditional treatments for oral cancer may lead to a broad spectrum of adverse effects to the maxillomandibular complex and associated structures which may demand an adjustment or discontinuation of the treatment. This review highlights the principle mechanism of action of photodynamic therapy (PDT), advantages, limitations and its amazing applications in the management of oral malignancies.

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INTRODUCTION

Oral cancer is the most prevalent forms of cancer in the world population.1 According to current estimates, about 275,000 new cases of oral cancer are diagnosed each year in the world.2,3 Frequently used therapies for the treatment of oral cancer include surgery, radiotherapy and chemotherapy.4,5 Traditional treatments for oral cancer may lead to adverse effects to the maxillomandibular complex and associated structures.6,7 Photodynamic therapy (PDT) is a relatively new minimally invasive method of treating a variety of tumors. The treatment can be delivered under local or general anesthesia, and the delivery method can include surface illumination or interstitial application. This therapy can be repeated as required as there is no cumulative toxicity; it can also be applied before or after any of the conventional treatment modalities.8 Von Tappeiner and Jodlbauer defined PDT as the dynamic interaction among light, a photosensitizing agent, and oxygen resulting in tissue destruction.9 John Toth wrote the first ‘white paper’ on ‘photodynamic therapy’ (PDT).10 In 1978 Thomas Doughetry carried out the first large experiments on humans.11 PDT was first approved by the Food and Drug Administration in 1999 to treat precancerous skin lesions of face or scalp.10 The overall morbidity and mortality after PDT is far less when compared with other conventional modalities.12

COMPONENTS OF PDT

The three fundamental elements of PDT are oxygen, a photosensitizing agent (PS), and visible light.8 PSs are selectively retained in tumor tissues and induce cytotoxicity after light irradiation.13 These agents are grouped under three generations. First generation include Photofrin (most extensively used) and hematoporphyrin derivatives (HPD). Second generation include 5-aminolevulinic acid (ALA), benzoporphyrin derivatives (BPD), m-tetrahydroxyporphyrin- chlorin (mTHPC) and talaporfin sodium (LS11). Foscam (mTHPC) is the most potent among them. Third generation PSs include currently available drugs that are modified by targeting with monoclonal antibodies or with nonantibody-based protein carriers and protein/receptor systems, and conjugation with a radioactive tag. Currently only three agents have been approved by FDA namely porfimer sodium, ALA and verteporfin. Foscam is in use only in European countries. Typical treatment times for first generation sensitizers are about 1,000 seconds; newer PSs can induce effective cell killing with the application of about 200 seconds of nonthermal laser light.14,15

LIGHT SOURCE

Red and infrared radiations penetrate more deeply into the tissues (Fig. 1). The region between 600 and 1,200 nm is often called the optical window of tissue. However, light up to only approximately 800 nm can generate $^1\text{O}_2$, because longer wavelengths have insufficient energy to initiate a photodynamic reaction.16 The choice of light source should therefore be based on PS absorption (fluorescence excitation and action spectra), disease (location, size of lesions, accessibility and tissue characteristics), cost and size. The clinical efficacy of PDT is dependent on complex dosimetry: Total light dose, light exposure time and light delivery mode (single vs fractionated or even metronomic). The fluence rate also affects PDT response.17

LIGHT SYSTEMS USED

1. Diode laser systems: They are easy to handle, portable and cost-effective, so are used predominantly.

2. Noncoherent light sources: Preferred for treatment of larger areas and include tungsten filament, quartz halogen, xenon arc, metal halide and phosphor-coated sodium lamps.
3. Nonlaser light sources include light emitting diodes (LED). They are economical, small, light weight and highly flexible.14,15,18

MECHANISM OF PDT

PDT is a multistep process involving the selective absorption of a photosensitizing drug by the tumor tissue, followed by the irradiation of the neoplastic lesion by a light source with specific wavelength. This radiation is capable of triggering photochemical reactions that generates singlet oxygen (1O2) and other reactive oxygen species (ROS). ROS generated by this process cause cytotoxic effects on tumor cells leading to tumor destruction. The drugs clinically used in PDT may bind the plasma membrane, intracellular membranes of the endoplasmic reticulum, mitochondria, lysosomes or combinations of these sites. Once PSSs do not have the nucleus is a primary site to bind, such anticancer therapy can be considered less genotoxic if compared with radiotherapy or chemotherapy.

Photodynamic effect can be demonstrated through two mechanisms called types I and II. The processes involved in both mechanisms are shown in the Jablonski diagram (Fig. 2). After absorbing a photon, the molecule of the PS changes from the ground state (S0) to the singlet excited state (S1). From this excited state, the PS can return to the ground state emitting a photon of energy through non-radioactive and radiative processes (fluorescence). The PS, in the excited state, can also reverse spontaneously its spin through the process of intersystem crossing and go from S1 to the triplet state (T1). Once formed, the T1 can undergo decay to the ground state via nonradioactive and radioactive processes (phosphorescence).19

The type I mechanism involves reactions of electron transfer between the molecule of the PS in their excited state S1 or T1 and the substrate. This process results in the formation of ion radicals that tend to react instantly with oxygen, producing a mixture of highly reactive oxygen intermediates like superoxide radical (•O2), hydrogen peroxide (H2O2) and hydroxyl radical (•OH), which oxidize a wide variety of biomolecules. The type II mechanism is characterized by reactions of energy transfer between the PS in the T1 state and molecular oxygen, which is also a triplet in the ground state (T0). These reactions lead to the formation of singlet oxygen (1O2), which is able to rapidly oxidize cell constituents and organelles resulting in the death of cancer cells. Both mechanisms may occur simultaneously. The proportion between them is highly influenced by the PS, the substrate, the oxygen concentration and the binding of PS to the substrate.20

The singlet oxygen produced by a photochemical reaction is a highly reactive species, with electrophilic character. This ROS is able to induce oxidation of cellular molecules. Protein and unsaturated lipids are their main targets, resulting in irreversible damage to cellular organelles and cancer cells death. This reactive form of oxygen can also cause damage to the tumor vasculature, resulting in an indirect form of tumor cell death, by hypoxia or starvation. Singlet oxygen has a short lifetime in biological systems (<0.04 ìs) and a diffusion potential with a small radius of action (0.02 ìm). Therefore, tissue damage resulting from photodynamic treatment is restricted to cancer cells and the penetration depth of light used to activate the PS.21

Dolmans et al (2003) describe that the generation of ROS leads to tumor destruction by three main biological mechanisms namely direct destruction of tumor mass by the action of ROS, damage to the tumor vasculature, creating areas of hypoxia in tumor masses and reduction of tumor mass in a secondary phase as a result of the activation of the immune system by necrosis and/or apoptosis.20

CYTOPROTECTIVE MECHANISMS

Cancer cells exploit cytoprotective mechanisms to avoid the cytotoxic effects of PDT.22 The first mechanism identified was based on the large variation observed in the level of antioxidant molecules expressed in cancer cells.23
Both water-soluble antioxidants [e.g. some amino acids, glutathione (GSH), or vitamin C] and lipid-soluble antioxidants (e.g. vitamin E) are present at variable levels in many cancer cell types, explaining the large variation in PDT sensitivity.24

A second mechanism is associated with expression in cancer cells of enzymes that can detoxify ROS. Although there is no specific cellular enzyme that can directly detoxify $^{1}\text{O}_2$, enzymes involved in other ROS metabolism can influence the cytotoxic effect of PDT. For example, superoxide dismutase (SOD) over expression or treatment with SOD mimetics have been shown to counteract the cytotoxic effect of PDT.25 An increase in SOD activity has also been observed in various cancer cell types after PDT, and this is associated with a decrease in GSH peroxidase and catalase activities.26 The third cytoprotective mechanism involves proteins whose encoding genes are themselves induced by PDT. Many categories can be specified but most of them are part of signaling pathways that can regulate PDT-induced apoptosis27 or participate in the repair of lesions induced by oxidative stress. NF-$\kappa$B inhibition by overexpression of the IKBa super-repressor or by the use of pharmacological inhibitors strongly sensitizes cancer cells to apoptosis induced by PDT.28

**PDT AND ORAL CANCER**

PDT has been successfully employed to treat early carcinomas of the oral cavity, pharynx and larynx, preserving normal tissue and vital functions of speech and swallowing.29,30 The ability to guide the treatment intraoperatively under ultrasound guidance allows it a leading role in interventional surgical oncology.12

Kawczyk-Krupka A et al subjected 48 patients with histopathologically confirmed oral leukoplakia to PDT in two groups. The first group consisting of 30 patients was treated with 20% ALA and light of a 630 nm wavelength from a Diomed laser and the second group consisting of 18 patients was subjected to 635 nm light generated from an argon laser with a PS of 10% ALA. The follow-up time was 4 to 34 months. In the first group a complete response was observed in 26 patients (87%), recurrence in four patients, whereas in the second group complete response referred to 16 patients (89%) and recurrence was observed in two patients.31

Betz CS et al in 2007 gave PDT with the application of Foscam to 13 patients with lymphangioma, hemangioma or neurofibromatosis. A significant reduction in pathological tissue volume with no injury to the covering skin was observed in every patient. There was no damage observed in the case of nerves or main blood vessels. Betz CS et al in 2007 conducted a study in 329 patients with head and neck tumors. The applied PSs were Photosens, Radachlorine and Alasens. With the application of Photosens in a dose of 0.4 to 0.8 mg/kg a complete response was achieved in 76% and partial response in 21.9% of the patients. In the case of Radachlorine administered in a dose of 1.2 to 2.4 mg/kg this effectiveness was 71.4 and 28.6% respectively. Very good results were gained in the case of patients with larynx and lower lip carcinomas.32

Real-time dosimetry with isotope detecting fibers creates possibilities for real-time measurement of the light penetration in the tissue. Blood oxygen level dependent (BOLD) MR can be also used as the marker of photodynamic activity. PDT is well tolerated by the nervous tissue causing minimal or even no deficit in its functionality. Vascular infiltration is also a rare problem and it can be eliminated by the application of covered endoluminal stents. In the majority of patients the necrosis of neoplastic tissue, regression of symptoms and prolongation of survival time are obtained.33 Also, unlike ionizing radiation, repeated applications of the PS and activating light treatments can be performed indefinitely.34

**NOVEL STRATEGIES IN PDT**

**Two-Photon PDT**

In 2-photon PDT, short (approximately 100 femtosecond) laser pulses with very high peak power are used, so that 2 light photons are absorbed simultaneously by the PS. Because each photon only contributes one-half of the excitation energy, near-infrared light can be used to achieve deeper tissue penetration. The subsequent photochemistry and photobiological effects are the same as in 1-photon PDT. Starkey et al reported 2 cm effective treatment depth in tumor xenografts; this is considerably greater than what would typically be achieved by 1-photon activation.35 Alternatively, if the laser beam is strongly focused, then the activation volume may be extremely small. This may be exploited to target individual blood vessels, reducing damage to adjacent tissues. Both approaches have used novel PSs designed to have very high 2-photon cross-sections.35,36

**Metronic PDT**

In metronic PDT (nPDT) both the drug and light are delivered at very low dose rates over an extended period (hours-days). This can result in tumor cell-specific apoptosis, with minimal tissue necrosis.37

**PDT Molecular Beacons**

The concept of PDT molecular beacons (MBs) derives from the use of MBs as fluorescent probes with high target specificity. The PS is linked to a quenching molecule, so that it is inactive until the linker is cleaved by a target...
specific enzyme (Fig. 3). Alternatively, the linker may be an antisense oligonucleotide (hairpin) loop, which is opened by hybridization to complementary mRNA. PDT MBs were first demonstrated using a caspase-3 linker between pyropheophorbide and a carotenoid quencher, achieving 8-fold and 4-fold quenching and unquenching, respectively, as demonstrated by the $^1O_2$ yield. Subsequently, matrix metallopeptinase (MMP)-based beacons were reported in vitro and in vivo, with high selectivity between MMP positive and MMP-negative tumors.

**Nanotechnology in PDT**

Nanoparticles (NP) have several potential roles in PDT: For PS delivery, as PSSs per se, and as energy transducers. Liposomal NPs are used clinically for delivery of the water insoluble PS verteporfin. The potential advantage of NPs is that a high ‘payload’ can be delivered and they can be ‘decorated’ with multiple targeting moieties, such as antibodies or peptides. Other approaches include biodegradable polymers and ceramic (silica) and metallic (gold, iron oxide) NPs; magnetic NPs, in which an applied magnetic field enhances localization to the tumor; and hybrid NPs that allow both PDT and either another therapeutic strategy such as hyperthermia or an imaging technique, such as MRI. NP delivery of 2-photon PSs has also been reported, because these typically have very poor water solubility.

**PHOTOCHEMICAL INTERNALIZATION**

Photochemical internalization (PCI) was specifically designed to enhance the release of endocytosed macromolecules into the cytosol. It is based on the use of PSs located in endocytic vesicles, as shown in (Fig. 4). The unique properties of the PCI process may be used to activate the therapeutics only in the light-exposed area while unexposed normal tissues are spared. PCI has been shown to increase the biological activity of several molecules that do not readily penetrate the plasma membrane, including type I ribosome-inactivating proteins (RIPs), immunotoxins, plasmids, adenoviruses, various oligonucleotides, dendrimer-based delivery of chemotherapeutics and unconjugated chemotherapeutics such as bleomycin and doxorubicin.

**ADVANTAGES OF PDT**

1. Is noninvasive and convenient for the patient.
2. Can be performed in outpatient or day-care settings.
3. Can be targeted accurately and selectively in early or localized diseases.
4. It cannot cure advanced disseminated disease but can improve quality of life and lengthen survival.
5. Repeated doses can be given without the need for total dose limitations.
6. Economical
7. Shows faster postoperative healing with no long-term side effects.
8. Can have excellent cosmetic results and the healing process results in little or no scarring.

**LIMITATIONS OF PDT**

1. Light needed to activate PS cannot penetrate more than 1.5 cm of tissue depth using standard laser and low powered LED technology and hence is less effective in treatment of large tumors and metastasis.
2. It may leave many people very sensitive to light post-therapy (photosensitivity).
3. Cannot be used in people allergic to porphyrins.
4. The lack of accurate dosimetry and suitable illumination devices has diminished the success of PDT.
APPLICATIONS IN DENTISTRY

Applications of PDT in dentistry are growing rapidly as in the treatment of oral cancer, bacterial and fungal infection therapies and the photodynamic diagnosis (PDD) of the malignant transformation of oral lesions. PDT has shown promise in the treatment of oral leukoplakia, oral lichen planus, and head and neck cancer. Photodynamic antimicrobial chemotherapy (PACT) has been efficacious in the treatment of bacterial, fungal, parasitic and viral infections. PDT also represents a novel therapeutic approach in the management of oral biofilms. Disruption of plaque structure has important consequences for homeostasis within the biofilm. Studies are now leading toward selective PSs, since killing the entire flora leaves patients open to opportunistic infections. Dentists deal with oral infections on a regular basis. The oral cavity is especially suitable for PACT, because it is relatively accessible to illumination.15

CONCLUSION

PDT is still considered to be a new and promising antitumor strategy. Its full potential has yet to be shown, and its range of applications alone or in combination with other approved or experimental therapeutic approaches is definitely not exhausted. It has also achieved significant clinical benefit and improvement in quality of life in patients with advanced oral malignancies. The advantage of PDT over the other conventional modalities of surgery, radiation and chemotherapy is that it is a minimally invasive treatment technique that lacks systemic toxicity yet results in selective tumor destruction with normal tissue preservation. This advantage is of particular importance for cancers of the head and neck, where excessive tissue loss results in significant functional deilities.

REFERENCES


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