APPLICATION OF THE PHOTODYNAMIC THERAPY IN MEDICINE AND DENTISTRY

Literature Review on Photodynamic and Antimicrobial Photodynamic Therapy

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Abstract: Photodynamic therapy (PDT) is recently being recognized as an attractive, non-invasive and alternative treatment method for precancerous lesions and superficial cancers. PDT has many advantages when compared with conventional treatment modalities. It has also been used for the photoinactivation of microbes. There is an increasing interest in the practical application of antimicrobial photodynamic therapy (aPDT) in many branches of dentistry, especially in periodontology, for the management of such conditions as chronic periodontitis or periimplantitis. The aim of the present paper was to discuss the application of photodynamic therapy in medicine and dentistry. The results of many so far published studies seem to be very promising indicating at the same time that further research is needed to establish the optimal protocol for effective photodestruction of tumor cells and microorganisms.

1 INTRODUCTION

Photodynamic therapy (PDT) is a medical treatment that utilizes light to activate a photosensitizing agent in the presence of oxygen. It is a noninvasive and painless medical procedure with relatively little side effects. Its use in medicine and dentistry is becoming widespread.

2 PRINCIPLE OF PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) involves three agents, i.e. photosensitizer, light and oxygen. The administration of a photosensitizer is followed by irradiation with the light of a specific wavelength (Takasaki et al., 2009). Upon photon absorption a molecule of the photosensitizer gets activated and transforms from its ground state (S_0) into an excited singlet state (S_1). The lifetime of the singlet state is in the nanosecond timescale (Stochel et al., 2009, chapter 17), which is too short to react with other molecules. From this state the drug may decay back to the ground state by emitting fluorescence or by internal conversion with energy lost as heat. However, to obtain a therapeutic photodynamic effect, the molecule of the photosensitizer must undergo electron spin conversion to its triplet state (T_1). The lifetime of the triplet state is in the microsecond to millisecond range (Soukos and Goodson, 2011). The molecule in its triplet state can again reach the ground state (in the case of light emission the process is called phosphorescence) or it can react further with oxygen according to two different types of mechanisms (Scheme 1) (Soukos and Goodson, 2011). Type I reaction involves electron-transfer reaction between the photosensitizer triplet state and a substrate (O_2). When oxygen participates in this process reactive oxygen species (ROS) (superoxide, hydroxyl radical, hydrogen peroxide) are produced. They are harmful to cell membrane integrity and cause irreparable biological damage. In the type II reaction the molecule of a photosensitizer in the triplet state transfers its energy directly to oxygen to form singlet oxygen ($^1O_2$) which is highly reactive and induces oxidative cell damage (Takasaki et al., 2009; Stochel et al., 2009, chapter 17; Soukos and Goodson, 2011).

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Scheme 1: Two types of mechanisms governing the photodynamic process. ROS are placed in rectangles; Ph – a photosensitizer in its singlet (S) or triplet (T) state.

Type I (electron transfer):

$$Ph \xrightarrow{h\nu} Ph^*(S) \longrightarrow Ph^*(T) \xrightarrow{O_2,\ H_2O} \longrightarrow Ph + \boxed{H_2O_2 + O_2^{\bullet-} + OH^{\bullet}}$$

Type II (energy transfer):

$$Ph \xrightarrow{h\nu} Ph^*(S) \longrightarrow Ph^*(T) \xrightarrow{^3O_2} Ph + \boxed{^1O_2}$$

2.1 PDT in the Treatment of Cancer

Photodynamic therapy is a relatively new treatment modality of localized cancers. Upon administration of a photosensitizer and its illumination, tumor cells are being directly killed as a result of oxidative damage (necrosis and apoptosis). Additionally, the vasculature of the tumor and surrounding tissues are damaged, resulting in indirect tumor cells death of hypoxia and starvation (Stochel et al., 2009, chapter 17); (Triesscheijn et al., 2006). The ideal photosensitizer for the use in oncology should possess the following properties: chemical purity, high binding affinity for tumor cells and low for host cells, non-toxicity in the dark, minimal risk of promoting mutagenic processes, high absorption coefficient within the phototherapeutic window (620-1000 nm) and as low as possible in the range of 400-600 nm to avoid skin sensitivity to solar irradiation after drug administration, high quantum yield of excited triplet state generation (the efficiency of PDT depends on photophysical properties of this state) (Stochel et al., 2009, chapter 17). Following photosensitizers are currently approved for the clinical use: Photofrin (porfimer sodium), Levulan (5-aminolevulinic acid), Metvix (methyl ester of ALA), mTHPC (meso-tetra-hydroxyphenyl-chlorin) (Triesscheijn et al., 2006). PDT had been applied clinically in the treatment of bladder cancer, skin cancer, Bowen's disease, head and neck cancer, esophageal cancer, Barrett's esophagus, endobronchial cancer, actinic keratoses (Triesscheijn et al., 2006); (Overholt et al., 2007).

In dental surgery, PDT has been applied in the treatment of oral leukoplakia, a premalignant lesion of the oral mucosa with a rate of malignant transformation of 0.1-17% (Spinola Ribeiro et al., 2010). Upon PDT with the use of ALA as a photosensitizer in combination with red light, all authors noted high response-to-treatment rate and a very low recurrence rate in a long-term observation (Spinola Ribeiro et al., 2010). Lin et al. (2010) reported excellent outcomes of PDT in the treatment of other oral precancerous lesions – oral verrucous hyperplasia (OVL) and oral erythroleukoplakia (OEL) (Lin et al., 2010). Upon the use of PDT with 20% ALA irradiated with 635 nm laser light, a complete response for 100% of OVL lesions and 95% of OEL was achieved after an average of 3.6 and 3.4 treatment sessions, respectively. The authors concluded, that for oral precancerous lesions ALA-PDT is one of the best treatments of choice.

2.2 Photodynamic Antimicrobial Chemotherapy (PACT)

The principle of PACT (also known as antimicrobial photodynamic therapy, aPDT) is similar to PDT. Photosensitizers and light (visible or UV) are used in order to induce phototoxic response, usually via an oxidative damage (Stochel et al., 2009, chapter 18). In PACT, the photosensitizer should basically possess properties similar to those expected for PDT, with a high binding affinity for microorganisms, broad spectrum of action and a low propensity for selecting resistant bacterial strains (Soukos and Goodson, 2011). The differences in susceptibility of gram-positive and gram-negative bacteria have been reported (Takasaki et al., 2009; Usacheva et al., 2001). Gram-positive bacteria are generally susceptible to photoinactivation. Gram-negative bacteria seem to be more resistant to PACT, mostly because of their additional outer membrane which decreases the permeability and reduces the photosensitizer uptake (Takasaki et al., 2009). Moreover, the surface of gram-negative bacteria cells is negatively charged, which makes anionic and neutral photosensitizers ineffective (Stochel et al., 2009, chapter 18). However, phenothiazinium dyes (methylene blue and toluidine blue), which are most commonly used in PACT, bear pronounced cationic charge and thanks to the electrostatic interaction can bind to the outer membrane of both gram-negative and gram-positive bacteria and penetrate bacterial cells (Soukos and Goodson, 2011); (Usacheva et al., 2001). Reactive oxygen species generated upon illumination of the photosensitizer are lethal to bacteria by oxidizing cell membrane (lipid peroxidation) causing its decomposition, followed by destruction of nucleic acids and proteins (Stochel et al., 2009, chapter 18).

2.3 PACT in Dentistry

2.3.1 Dental Caries

Dental caries is the result of tooth-hard tissue demineralization in the presence of acids secreted by supragingival biofilm bacteria (*Streptococcus* and

Lactinobacillus species) (Soukos and Goodson, 2011). Up to 10-fold reduction of the viability of *S. mutans*, the main cariogenic bacteria, was achieved by toluidine blue mediated PACT, even when the organisms were embedded in a collagen matrix mimicking carious dentin (Burns et al., 1995). The susceptibility of cariogenic bacteria was confirmed by other authors (Williams et al., 2004). PACT may be useful in the prevention of caries, management of early carious lesions and disinfection of carious cavities before restoration.

2.3.2 Endodontics

The success of the endodontic treatment relies on the elimination of infection from the root canal system. The conventional means to achieve it is to perform chemo-mechanical debridement and irrigation with disinfectant solutions, like sodium hypochlorite (NaOCl). However, anatomical complexity of the root canal system (isthmuses, ramifications, presence of dentinal tubules) makes complete removal of bacteria with standard procedures and medicaments almost impossible (Soukos and Goodson, 2011). Therefore, the adjunctive antimicrobial PDT (aPDT) has been employed to eliminate residual root canal bacteria in many studies, the results of which seem to be very promising. The combined use of red light and methylene blue results in reduction of *Enterococcus faecalis* viability by 40 – 97% in the experimentally infected root canals of extracted human teeth (Foschi et al., 2007; Silbert et al., 2000; Soukos et al., 2006). The results of *in vivo* studies conducted by Bonsor, Nichol, Reid and Pearson (2005 and 2006) point that PACT is as effective in root canal system disinfection as conventional chemo-mechanical techniques (instrumentation with NaOCl/citric acid irrigation) (Bonsor et al., 2005; Bonsor et al., 2006). These authors highlighted also, that aPDT is more biocompatibile than conventional irrigants. It was confirmed by Xu et al. (2009), who reported that although some of the light energy applied to the root canal escapes from the root apex (<10%), methylene blue-mediated aPDT is harmless to osteoblasts in the periapical region. This is not the case for sodium hypochlorite, which is highly toxic and damages cells of the periapical tissues (Xu et al., 2009).

2.3.3 Periodontology

Periodontology deals with the diseases of periodontium (gum, alveolar bone and periodontal ligament). Chronic periodontitis, the most common periodontal disease, which refers to approximately 48% of the population (Albandar, 2005) and is a major cause of tooth loss (Bakrami et al., 2008), is characterized by a progressive destruction of the periodontium's fibers and alveolar bone, resulting in following clinical symptoms: pathological pockets or gum recessions, attachment loss, bony defects, bleeding, hypermobility of the teeth and eventually tooth loss. Following gram-negative anaerobes are considered the most harmful for periodontium and are isolated from the deepest periodontal pockets and sites with severe bone loss: *Porphyromonas gingivalis*, *Tanerella forsythia* and *Treponema denticola* (so called 'red complex' according to Socransky) (Socransky and Haffajee, 2002).

The effective treatment of the periodontal disease is of a great importance also when general health is considered, as the relationship between periodontal disease and several systemic disorders, e.g. cardiovascular disease, diabetes mellitus, rheumatoid arthritis, cerebral infarction or hypertension was proved (Detert et al., 2010; Seymour et al., 2003; Lagervall et al., 2003). Effective bacteria eradication is the basis of periodontal treatment. Standard non-surgical treatment procedures, like supra- and subgingival plaque removal, have to be accompanied with some additional antimicrobial means, like the administration of antibiotics. However, the use of antibiotics, delivered systemically or locally, apart from many other side effects, promotes the emergence of resistant bacterial strains, which, according to WHO, is becoming a threatening problem in healthcare worldwide. From this standpoint, new and effective antimicrobial approaches are urgently needed to be introduced. The interest in use of PACT in periodontology is considerable. Its effectiveness against periopathogens has been proved in many *in vitro* studies: with the use of toluidine blue (TBO) or methylene blue (MB) as photosensitizers and a light wavelength of approximately 632 nm emitted by a He-Ne laser, significant reductions in the viability of bacteria were observed (Bhatti et al., 2002; O'Neil et al., 2002; Chan and Lai, 2003). Matevski et al. determined optimal PACT parameters for the effective photoinactivation of *P. gingivalis* in terms of light intensity (25 mW/cm^2), light dose (10 J/cm^2) and TBO concentration (12.5 µmol/ml) and applied them for inactivation of *P. gingivalis* resuspended in blood or serum to mimic actual periodontal pocket conditions. Interestingly, in the presence of blood or serum, the decline in bacteria viability was still statistically significant, but there was a large decrease in effectiveness compared with

blood/serum-free suspensions. Blood and serum appeared to partially protect *P. gingivalis* from PACT. This effect can be explained by a lowered light penetration through blood and serum (due to light absorption and scattering by these media) and by scavenging of photogenerated reactive oxygen species through oxidation of blood/serum organic components.

The susceptibility of *P. gingivalis* to PDT was also confirmed in animal model study conducted by Koemerik et al. (2003). Upon the use of 1 mg/ml of TBO in combination with increasing light doses (6, 12, 24 and 48 J) in rats previously infected with *P. gingivalis*, no viable bacteria were detected. After irradiation, histological examination was carried out. No adverse effects of PACT on the periodontal tissues were observed. Even with the highest concentration of TBO (1 mg/ml) and the highest light dose tested (48 J) no ulcer on epithelium or inflammation of the connective tissue were detected. The authors evaluated the alveolar bone levels of the maxillary molars by morphometric and radiographic methods. The results showed that with the use of TBO concentration of 0.1 and 1 mg/ml in combination with 48 J of laser light, the bone loss was significantly reduced in comparison with the control group that did not receive PACT (Koemerik et al., 2003). The biodistribution of topically applied TBO on the gingival tissues was also examined. It was demonstrated that the photosensitizer penetrated throughout the epithelium. This fact may have very advantageous clinical implications, as conventional periodontal debridement fails to eliminate pathogenic bacteria that are placed in the soft tissues. In the study conducted by Fernandes et al. (2009) PACT was applied as an adjunctive treatment to scaling and root planning (SRP) to immunosuppressed and non-immunosuppressed rats with experimentally ligature-induced periodontitis in mandibular molars. In rats that received PDT, the periodontal ligament was found to be intact, with parallel collagen fibers, lack of an inflammatory infiltrate and thick alveolar bone, which was not the case for rats treated only with SRP or SRP and TBO with no irradiation (Fernandes et al., 2009).

The outcomes of *in vivo* studies are, however, divergent. Some authors reported that adjunctive PACT has a positive effect on periodontal parameters contributing to the statistically significant decrease of bleeding and probing depths and gain of clinical attachment in comparison with conventional treatment (SRP) (Braun et al., 2008). In comparison, Polansky, Haas, Heschl and Wimmer (2009) concluded, that PACT does not provide additional benefits to conventional periodontal treatment, although visibly larger reductions of bleeding indices were seen among the patients that received PACT than in the control group, however these differences turned out to be statistically insignificant (Polansky et al., 2009). Similar results were obtained by other authors (Chondros et al., 2009). The differences in the outcomes of *in vitro* and *in vivo* studies indicate that more detailed research is needed in this field.

2.3.4 Periimplantitis

Periimplantitis is an inflammatory condition that affects soft and hard tissues surrounding an osseointegrated dental implant and may lead to its failure. The causative flora is similar to that one responsible for the development of periodontal disease (*A. actinomycetemcomitans, P. gingivalis, P. intermedia*) (Takasaki et al., 2009). In an animal split-mouth study, Shibli et al. (2006) compared histometrically the outcomes of conventional periimplantitis management (debridement + guided bone regeneration) with those of conventional management combined with TBO-mediated PACT in dogs with ligature-induces periimplantitis. The use of PACT resulted in a greater bone gain – the mean percentage of re-osseointegration was 31-41% for the test group and 0-14% for the control group (Shibli et al. 2006). Haas, Baron, Doertbudak and Watzek (2000) used TBO-mediated PACT in combination with soft laser (906 nm) as an adjunct to autogenous bone augmentation in 17 patients with periimplantitis. The mean radiographic bone gain 4 months after the procedure was 2 mm (maxilla – 2.5 mm; mandible – 1.9 mm), what can be considered as an excellent clinical outcome (Haas et al., 2000).

2.3.5 Soft Tissue Therapy

The effectiveness of PACT in the treatment of recurrent herpes labialis (RHL) was also investigated (Sperandio et al., 2009). Great clinical outcomes were achieved for treating already established RHL vesicles, compared to conventional treatment with the use of antiviral compounds. Patients reported an immediate pain relief after the procedure. No recurrence was observed in a 6-month period (Sperandio et al., 2009).

2.4 Towards Increased Effectiveness

The reduced susceptibility of *P. gingivalis* and other periopathogens to PACT *in vivo* can be explained by the fact, that periodontitis is a biofilm-related

infection. The penetration of the photosensitizer solution into the bacterial biofilm is decreased in comparison to the suspensions of bacteria used in *in vitro* studies. Therefore, to enhance the effectiveness of PACT, the development of novel delivery and targeting approaches may be required. One strategy to improve the targeting was proposed by Bhatti et al. (2000). The authors used a conjugate of TBO and murine monoclonal antibody (Ab-TBO) to specifically target *P. gingivalis* in the presence of *S. sanguis* or human gingival fibroblasts (HGFs) *in vitro*. It was demonstrated that with the use of Ab-TBO conjugate a high selectivity and efficiency in the killing of *P. gingivalis* can be achieved. Such an approach could enable the killing of important periopathogens without collateral damage either to host tissues or to the normal oral microflora.

3 CONCLUSIONS

PDT and PACT are non-invasive, relatively inexpensive, painless to the patient with little or no side-effects. The outcomes of presented *in vitro* and *in vivo* studies are very promising. However, more research is still needed in this field for optimizing the protocol of clinical application, improving specific targeting of tumor cells and bacteria and introducing new groups of photosensitizers.

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