Chapter 6

THE IMPORTANCE OF PHOTODYNAMIC THERAPY IN DENTISTRY

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Photodynamic Therapy

Photodynamic therapy is defined as the termination of the viability of target cells by generating reactive oxygen species in the presence of a photosensitizing agent with an appropriate wavelength and dose of light.[1,2]

The foundations of modern phototherapy were laid at the beginning of the 19th century. Oskar Raab proved that some chemical compounds such as acridine and eosin have toxic effects under light.[2]

The biggest step in the destruction of living microorganisms by light was taken with the production of the photosensitizing substance called Photofrin and the treatment protocol in many countries including the United States of America. This approach, which is effective on many microorganisms such as bacteria, viruses, protozoans and fungi, is particularly effective in localized and superficial infections.[3] For this reason, photodynamic therapy, also called "Photodynamic Antimicrobial Therapy", is a potential treatment approach in the field of dentistry, especially for infections in the oral cavity such as mucosal and endodontic infections, periodontal diseases, caries and periimlantitis.[1, 2, 4]

It is reported as a promising treatment because it is localized and non-invasive. While bacteria cannot develop resistance against photodynamic therapy, the application has no genotoxic or mutogenic effect.[5] It has been reported that the treatment, which is effective on both Gr (+) and Gr (-) bacteria, has a higher effect especially on Gr (+) bacteria.[5]

Mechanism of Action of Photodynamic Therapy

The basic principle of photodynamic therapy is the "killing effect" that occurs by a series of mechanisms. Photodynamic therapy includes three components: photosensitizer, light and oxygen.[1, 6] Photosensitizers are light-sensitive agents that absorb visible light, causing electron transfer or transferring light energy to its environment. In the presence of oxygen, they cause oxidative reactions that are toxic to microorganisms.[7] Toxic products resulting from a series of photochemical reactions occurring in the presence of photosensitizers with the effect of light at the appropriate wavelength and dose cause target cell death through oxidative damage.[1,8]

The first step of photosensitization reactions is the absorption of photon energy from the light source by the photosensitizer. Photosensitizers have a stable electronic configuration at the lowest energy level. When the light sensitive drug is exposed to light energy in the target area, the energy level rises after some energy distributions.[1, 9]

The photosensitizer, which absorbs light at the appropriate wavelength, changes from the singular state having a low energy level to the excited singular state. Later, the photosensitizer can return to singular state as a result of the emission of light from the excited singular state, or it switches to the excited triple state with a high energy level. The stimulated triple state causes rapid and selective destruction in the target tissue by creating singular oxygen and other free radicals with high reactivity. Two mechanisms occur in the interaction of the photosensitizer with the biomolecules of the excited triple state.[1, 2]

In the Type I reaction, free radicals are formed by direct electron / hydrogen transfer from the photosensitizer, formation of ions, redox reactions or electron / hydrogen separation from the substrate molecule. As a result of the rapid reaction of these radicals with oxygen, reactive oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide are formed.

In the Type II reaction, the photosensitizer is electronically stimulated as a result of transferring the energy of its excited ternary form to molecular oxygen and single oxygen, known as the highly reactive form of oxygen, is formed. Photodynamic therapy is also very difficult to distinguish between these two mechanisms. However, it should be known that the damage caused by both mechanisms depends on the oxygen and the concentration of the photosensitizer.

Type II reactions are considered to be the main mechanism leading to photooxidative microbial cell damage. The photodynamic effect describes type II photoreactions that are mainly dependent on oxygen.[1, 2, 7, 10]

Sensitivity to photodynamic therapy varies depending on the cell wall structures of bacteria. Photosensitizer penetrates sensitive areas inside the cell thanks to the peptidoglycan and lipoteiotic pores in the cytoplasmic membrane of Gram positive bacteria. The outer membrane of Gram-negative bacteria acts as a physical and functional barrier between the cell and its external environment.[1, 11, 12]

Photodynamic inactivation can be achieved in both gram-positive and gram-negative bacteria with cationic photosensitizers.[12] In Gram-negative bacteria, the strong negative charge arising from the lipopolysaccharide layer on the outer membrane prevents neutral or anionic photosensitizers from entering the gram negative bacterial cell.[9]

Neutral or anionic photosensitizers are effective only on gram positive bacteria.[9,12]

Photodynamic therapy in bacteria creates a cytotoxic effect by affecting intracellular organelles and biomolecules through photodamage. Mitochondria, lysosome, cytoplasmic membrane and nucleic acid are potential targets for photodynamic therapy.[1] Two mechanisms are proposed regarding the cellular damage caused by photodynamic therapy. The first is DNA damage, the second is cytoplasmic membrane damage or inactivation of cell membrane permeability and enzymes.[13]

The individual oxygen and free radicals that emerge as a result of photodynamic therapy affect many cell structures and show their effectiveness by following different metabolic pathways. In the use of antibiotics, resistance to this antibiotic may develop over time, while microbial cells cannot develop resistance to Photodynamic therapy.[1, 2]

Another advantage of photodynamic therapy over antibiotics is that it does not cause the secretion of proinflammatory cytokines. It has been observed that the damage to the cell membrane with photodynamic therapy is through lipid peroxidation and protein damage.[13]

However, antibiotics cause the development of a series of pathological events that result in tissue damage by causing the release of proinflammatory cytokines from cells.[14]

Photosensitizing Substances

The substances that initiate the photochemical reaction are photosensitizers and light sensitive agents. Photosensitizers, one of the three components of photodynamic therapy, absorb light and create a toxic effect for the cell. Thousands of photoactive compounds consisting of various natural and synthetic substances are known as photosensitizers. An ideal photosensitizer should be non-toxic, show local toxicity and be activated only under light.[1-3]

More than 400 compounds consisting of dyes and various natural substances are known as photosensitizers. The agents to be used in the treatment of microbial infections are required to have sufficient photophysical (maximum absorption wavelength, light absorption amount) and physicochemical (lipophilicity, ionization) properties, and to create a high amount of long-lasting oxygen. These agents, while killing microbial cells, are expected to cause minimal damage to host tissues and prevent the reproduction of pathogenic microorganisms after treatment. In addition, an ideal Photosensitizer should be stable during storage and application.[14]

Photosensitizing agents most commonly used in dentistry; toludine blue, methylene blue, rose bengal, malachite green and hypericin. However, with the increasing interest in natural materials in the new period, dyes obtained from natural plants; Hypericum perforatum, curcuma longa (turmeric) and oligomeric proanthocyanidins have been tried and successful results have been obtained.

Toluidine Blue

Toludin blue and methylene blue, which have the same chemical properties, are often used in antimicrobial photo-active disinfection. Toluidine blue is a basic drug that has been used in the antimicrobial field for many years. It is a blue cationic dye. Toluidine has the ability to kill a wide range of bacteria, including blue methicillin-resistant Staphylococcus aureus. Toluidine blue shows its effect by producing singular oxygen under light in the wavelength range of 630 nm.[7, 16]

Toluidine blue can be applied topically in the treatment of oral infections. In topical application, it has been shown that toluidine blue penetrates all layers of the epithelium, but in amounts negligible to the connective tissue. Toludin blue is a thiazine dye from the quinone-imine family. Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Fusobacterium spp. and more effective on lipopolysaccharides.[17]

Hypericin (Hypericum perforatum L.)

It is thought that Hypericin, which is naturally obtained from plants of the Hypericum type, is the strongest Photosensitizing agent found in nature. Hypericin is a phenanthroperylene quinine pigment naturally found in plants and is a natural photoactive. Hypericin can be synthesized naturally from plants of the hypericum type and is activated at 590-600 nm.[18]

Hypericum perforatum L. (Hypericaceae) plant has been used since ancient times for its antidepressant properties. It is also known to speed up wound healing. There are creams, ointments and extracts of the plant containing phenylpropane, flavanol derivatives, biflavones, proanthocyanidins, xanthons, chloroglycins, some amino acids, naphthodyantrons and essential oils. Two compounds called hypericin and hyperforin can be synthesized from the plant.[18]

Rose Bengal

Rose bengal is a structure with an oxygen atom in the middle of three aromatic rings and generates long-lived radicals. It is a xanthene dye that is activated at 450–600 nm. Rose bengal is an anionic compound especially effective against gram (+) bacteria.[19]

Rose can form singular oxygen, known as the highly reactive form of bengal oxygen. The high rate of halogen in the structure of rose bengal and its high molecular weight in these halogens enable this Photosensitizer to be more effective than other halogenated derivatives. This is because the increase in the amount of halogen and the fact that the photosensitizer contains higher halogens by weight facilitates the formation of an induced triple state that will generate high reactivity single oxygen and other free radicals that cause rapid and selective destruction of the target tissue.[20]

Malachite green

The dye from the triarylmethane family absorbs red light. The material, which is mostly studied in periodontology, has been shown to be effective on Staphylococci, Enterobacteria and Candida with the study of Junqueira et al. (2010).

Proanthocyanidins

Proanthocyanidin, also known as procyanidin, is a group of polyphenolic compounds found naturally in fruits, vegetables, nuts, seeds, and flowers.[21]

In 1987, thanks to Jack Masquelier's powerful free radical scavenging ability at the same time obtaining oligomeric proanthocyanidins from apples, blueberries, avocado seeds, immature cocoa beans, horse chestnut, hawthorn fruit, birch flower, immature strawberry / blackberry, grape seed and red wine. It has been reported that proanthocyanidins claim to have an important therapeutic effect. The phenolic hydroxyl group in its structure acts as a hydrogen donor, effectively scavenging free radicals. It has been recognized to be effective in preventing many diseases such as hypertension, allergies, cardiovascular diseases and various infections. It is also thought to be anticarcinogenic, anti-inflammatory and vasodilator.[21,23]

Turmeric (Curcuma longa L.)

Turmeric (Curcuma longa L.) is one of the most recently prominent plant-derived Photosensitizer substances. The extract of the plant, which was used as a medicine in liver diseases, in the treatment of inflamed joints and wound healing in ancient times, is yellow pigmented and activated at 430 nm.[14, 22]

However, the low water solubility capacity of turmeric has been reported as a disadvantage. [23]

Rumex cristatus DC. (Labada)

Plants are used for medicines or dietary supplements due to their antioxidant, anti-inflammatory and antimicrobial properties. It is a plant from the Polygonaceae family, with more than 200 varieties all over the world, twenty three of which are found in Turkey. It has been used in traditional medicine for its anti-inflammatory and constipating effect. As a result of phytochemical screening, Labada derivatives were found to contain flavonoids, terpenes, organic acids and naphthalene.[24]

Light in Photodynamic Therapy

Photodynamic therapy requires a low-power visible light source that is compatible with the absorption spectrum of the Photosensitizer and can activate the Photosensitiser.[1]

Photosensitizers used today are activated with red light between 630-660 wavelengths.[17] Lasers, which are used as a standard light source in photodynamic therapy, provide superiority to ordinary light because of their ability to provide high energy, produce monochromatic light, and ease of access to the desired area by means of fiber optics. Complicated dye laser systems were used as a light source before, but in recent years, diode lasers, which are portable, much cheaper and extremely easy to use, have been developed. In addition, non-laser light sources have come into use.[17]

The wavelength of the light used and the activation peak of the Photosensitizer must be compatible with each other.[25] In this sense, the use of LED and filter lamps with a wide wavelength range in this area will allow the activation of many Photosensitizers. [26] In photodynamic therapy, various high-energy filter lamps are used in the clinical environment other than laser sources.[25] Light sources such as tungsten filament, quartz halogen, xenon arc and phosphor-coated sodium lamps are used in the treatment of large areas.[1]

Although these light sources are inexpensive, their general maintenance is also easier. Compared to lasers, filter lamps have a much wider wavelength range. Combined with various filters, they emit light of specific wavelengths. However, this situation causes a decrease in light power. Therefore, its use is limited in skin lesions.[25]

LEDs and halogen light sources, which are routinely used in dentistry, also constitute the basic light sources to be used in the activation of the Photosensitizer.[12] Halogen light sources have been used in dentistry for many years for the polymerization of fissure sealants, bonding agents, resin cement and composite resins. It is sufficient that the wavelength of the light used to polymerize these materials is around 400-500 nm.[27]

With the development of LEDs in recent years, these light sources have started to be used in Photodynamic therapy. LEDs, which have various advantages in clinical environment, provide ease of use in difficult to reach anatomical areas.[25] Although these light sources are cheaper and smaller, they are also lighter and easier to use.[1] Wavelength range ranges from 350 nm to 1100 nm.[25] In a study by Lima et al.,(2009) microorganisms in dentin caries were eliminated by activating the photosensitizer using an LED device.

Photodynamic therapy also depends on the depth (penetration) the light can reach in the tissue, the wavelength of the light used, and the penetration of the light into the tissues increases as the wavelength of the light increases. Although red light is mostly used in photodynamic therapy, the intensity of this light decreases in the tissue and can penetrate up to 1 cm. When near infrared light (700-850 nm) is used, tissue penetration approximately doubles. For this purpose, the development of new Photosensitizers that can be activated at this wavelength continues.[2, 3, 17]

Oxygen in Photodynamic Therapy

For photodynamic therapy to take place, the presence of oxygen is required and the inactivation of microorganisms occurs by photo-oxidation of biomolecules in cells.[28] In the type I reaction that occurs after the interaction of the triplet form of the photosensitizer with biomolecules, direct electron / hydrogen transfer from the photosensitizer results in the formation of ions, redox reactions, or the formation of reactive oxygen species such as hydroxyl radicals, superoxide and peroxide anions by the formation of electron / hydrogen from the substrate molecule. In the type II reaction, single oxygen, which is electronically excited and known as the highly reactive form of oxygen, is formed. The damage caused by these two reactions in the target tissue depends on both the energy level of oxygen and the concentration of photosensitizer.[1, 2]

The primary toxic product involved in the type II reaction is single oxygen. Single oxygen causes microbial cell death by acting on viruses, fungi, protozoa and bacteria. In addition, while anti-oxidant enzymes such as superoxide, dismutase and catalase can protect the cell against some oxygen radicals, they cannot protect it against singular oxygen.[1, 8, 29]

Dentistry and Photodynamic Therapy

The oral cavity is complex, relatively specific, and contains gram-positive and gram-negative bacteria, fungi, mycoplasma, protozoa and viruses, which are highly associated with microorganisms.[1,12]

Bacteria, fungi, viruses and single-celled microorganisms can be eliminated by photodynamic therapy by creating singlet oxygen derivatives. Photodynamic therapy is recommended as an alternative or addition to existing disinfection methods in root canal treatments and treatment periodontal issues. The biggest advantage of using photodynamic therapy in endodontic treatments and periodontal treatments are that it eliminates harmful microorganisms without damaging the cells in the surrounding tissues and is successful even against bacteria that have developed resistance to many drugs.[2-4]

Biofilm is a complex organization formed by microorganisms in the extracellular matrix that live together in a certain structural integrity by adhering to the tooth surface and communicate with each other to fulfill the functions necessary for the continuation of their existence. Photosensitizer, which has direct effect on extracellular molecules, shows its antimicrobial effect through singular oxygen with high chemical reactivity. The fact that the photosensitizer is also effective on the polysaccharides in the extracellular matrix of the plaque is among the advantages of Photodynamic therapy compared to antibiotics. In this way, resistance developed against antibiotics against Photodynamic therapy, which inhibits plasmid change by breaking down the biofilm, does not develop.[1, 2, 4]

As is known, antibiotics show their effects by damaging certain parts of microorganisms. In contrast, Photodynamic therapy causes destruction in more than one part of the cell through oxidative damage. For this reason, it is very unlikely that all damaged areas gain resistance to Photodynamic therapy.[12, 13] Photodynamic therapy, which targets microorganisms in plaque in dentistry, can be used as a preventive method and as a noninvasive method by eliminating bacteria in the carious cavity. Photodynamic therapy, which performs a rapid bacterial death after a short light activation process, also has a short half-life of reactive oxygen species and a limited diffusion distance. In addition, the irradiation area is limited. This enables Photodynamic therapy to be applied in a specific area.[1, 30]

It is a matter of discussion that bacteria may remain inactive in the area where dentin is minimally affected in deep caries. In this context, using Photodynamic therapy to take advantage of its lethal effect on bacteria may be an appropriate approach.[4, 31]

There are many studies on oral microorganisms. It was found that toluidine blue applied to dentin caries created in situ by Lima et al. (2009) was effective on microorganisms and it was concluded that Photodynamic treatment would be a useful approach that can be used to eliminate microorganisms in dentin caries before restoration. In another study, photogem, hematoporphyrin and toluidine blue applied on L. acidophilus and S. mutans were activated with an LED light source and toluidine blue was reported to be the most effective photosensitizer among these three materials.[32]

Hypericin and Foslipos (FOS) were applied on S. mutans and S. sobrinus. In this study, halogen light source used for polymerization in dentistry was used to activate Photosensitizer and effective results were obtained.[33]

It is a promising treatment option due to the lack of resistance of bacteria against photodynamic therapy and also because it is localized and non-invasive.

In order for photodynamic therapy to be a reliable alternative to conventional antibiotics, the destructive effect of photodynamic therapy should be minimal, especially on tissues damaged by infection. Therefore, it is necessary to evaluate the results of well-planned controlled clinical studies in order to determine the optimum conditions.

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Chapter 7

PROTECTIVE EFFICACY OF NOBILETIN AND PHYSIOLOGICAL PATHWAYS IT USES

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